Identification of novel candidate genes involved in resistance against a fungal pathogen in *Drosophila melanogaster* using the DSPR and QTL analysis

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Abstract:

The severity of infection and its effect on organisms differs and has a genetic basis. Though many genes are known to directly play a role in immune pathways, more genes involved in immunity (including those that affect immunity in indirect ways or have multiple functions) are yet to be discovered. I used the model organism, *Drosophila melanogaster* to determine the genetic factors underlying survival after inoculation by the fungal pathogen, *Beauvaria bassiana*. A common use for *B. bassiana* is as a pest control agent in agricultural applications. In this experiment, I used the Drosophila Synthetic Population Resource (DSPR), a series of recombinant inbred lines (RILs) derived from an advanced cross of founder inbred lines. The resistance phenotype was quantified as the proportion of individuals surviving 10 days after infection. I identified 18 quantitative trait loci (QTLs) that are possibly associated with surviving infection. The candidate genes containing those QTL are involved with immunity, gene regulation, metabolism, and development/neural maintenance. Many of the genes also had orthologs with human genes involved in similar roles. My experiments suggest that the immunity and neural pathways may be more integrative than previously known. Further, my analyses revealed the possible role of regulatory genes in the modulation of resistance and survival during infection.

Key words: immunity, genes, DSPR, QTL, resistance, fungal pathogen, infection, survival

Introduction:

Infectious agents invade other organisms in an attempt to live off of their host's resources, reproduce, and/or take shelter. Through the process of infection, the host may experience symptoms related to disease and eventually, death. The immune system protects organisms from these pathogens and the damage that they cause. *Beauveria bassiana* is an entomopathogenic fungus that infects various insect hosts. *B. bassiana* has been used as a biological control agent for insect pests and vectors of human disease, such as mosquitos (Blanford et al., 2005). The fungus infects insects

through direct contact with the exoskeleton, piercing the cuticle and growing inside of the host (Howard et al., 2010). However, *B. bassiana* kills its hosts much more slowly than other insecticides (Howard et al., 2010). Because of this, most flies capable of reproducing will still contribute offspring to the next generation despite their infection phenotype in the long run, prohibiting the prompt selection of resistance or tolerance. Little is known about potential genes or pathways involved in resistance against *B. bassiana* and other fungal pathogens. Understanding which genes impact resistance in insects can help to clarify the complexity of resistance against pathogens in humans. *Drosophila melanogaster* serves as a good model organism for the study of genes, their functions, and their interactions. It is of utmost relevance since *D. melanogaster* has many genes and regulatory/metabolic pathways in common with those of humans, with these similarities having been used to research cancers, development, and organ function (Baker and Thummel, 2007).

It is well understood that immunity is shaped by selection and competition between host and pathogen (Lazzaro and Little, 2009). Genes having an effect on immunity should have a high heritability and should be identifiable in genetic studies (Craen et al., 2005). Attempting to identify novel immune genes in *D. melanogaster* requires the presence of abundant genetic diversity as well as variation in infection resistance phenotypes. Recombinant inbred lines (RILs) that originate from a synthetic founder population can be used to assess which genetic factors contribute to complex traits (Rose et al., 2011). I used the Drosophila Synthetic Population Resource (DSPR), which consists of ~1700 RILs derived from 15 founder inbred lines. These 15 founder RILs were separated into two populations of 8 founders each (with one founder line overlapping between populations). These populations were allowed to breed for 50 generations, allowing for highly recombined and intercrossed RILs (King et al., 2012). In effect, each RIL possesses a genetic mosaic of the founder lines, giving them great genetic and phenotypic diversity. Most importantly, the DSPR have been fully sequenced, which provides high-resolution maps of each RIL (King et al., 2012). Therefore, by phenotypically assaying the DSPR's resistance to *B. bassiana* infection, a Quantitative Trait Locus

(QTL) analysis can be performed to high resolution to identify possible key genetic regions underlying immune resistance. Using >800 lines of the DSPR, genome scans can have 84% power with a resolution of 1.5 cM (King et al., 2012). The DSPR and QTL analysis have been used to identify key genes involved in methotrexate toxicity in Drosophila as well as their homologs in humans (Kislukhin et al., 2013). The DSPR, paired with QTL analysis, is best equipped to identify alleles of low frequency with large effects (e.g. Rose et al., 2011).

This project uses the DSPR to identify crucial genes involved in *Drosophila*'s resistance to infection by *B. bassiana*. Initially, DSPR lines were inoculated with different doses of *B. bassiana* and survival was monitored until 100% death. With this information, I performed infection and resistance assays for 125 RILs, 22 of which I did in triplicate. A genome scan based on percent survival at day 10 revealed 18 loci possibly associated with immunity and survival. Investigations of genes at these positions revealed functions that previous research has shown to be involved in immunity, metabolism, homeostasis, and gene regulation. My research therefore provides an initial basis for testing novel genes that may impact the resistance phenotype.

Materials and Methods:

Maintenance of RILs (Experiment 1)

Twenty-three RILs of the DSPR were obtained from the University of California Irvine. RILs were reared and maintained on Cornell Biotech Glucose Drosophila Media (per liter of deionized water: 82 g glucose, 82 g Brewer's yeast, 10 g agar, 10 mL acid mix composed of 4.15% phosphoric acid by volume and 43.15% phosphoric acid by volume). 5 vials per line were reared at a time and kept on the same developmental cycle. The RIL stocks were maintained at 25° C on a 12hr light/12 dark cycle. 18 lines were selected at random and prepared for the actual experiments. 100 males and 100 females per line were anesthetized on CO_2 and placed into small fly cages with a small petri plate with fly media and yeast. The flies were allowed to lay eggs for 1-2 days and these eggs were

then divided into vials in groups of 60-80 eggs per vial. After 17 days from egg, adults were ready for the inoculation sprays (described below).

Maintenance of RILs (Experiment 2)

~300 RILs from the A1 population of the DSPR were obtained from the University of California Irvine. These were obtained independently and at a later date from the 23 RILs used in Experiment 1. The RILs were reared and maintained on Cornell Biotech Drosophila Media. Two copies of each RIL were maintained in vials, with RILs divided into groups, with the developmental stages staggered per group. The RIL stocks were maintained at 25° C on a 12hr light/12 dark cycle.

Preparation of the DSPR RILs for inoculation

2-3 day old adult flies were placed in a small, breathable plastic container containing a small petri plate with Biotech Drosophila media and yeast paste. The adults were allowed to lay eggs for one to two days. Then, the eggs were collected and placed in vials in groups of 60-80 eggs/vial to develop into adults.

Maintenance of B. bassiana

Beauveria bassiana ARSEF 8246, a shore-fly isolate from the United States Department of Agriculture on the Cornell campus, was passed through *D. melanogaster* for one generation. The spores that resulted were grown on petri plates of fungus growing medium (per 1L of deionized water: 10g glucose, 2.5g autolysed yeast extract, 2.5 g bactopeptone, and 15g agar). This new *B. bassiana* strain was stored at -20° C as “ARSEF 12460 Shahrestani & Vandenberg”.

Inoculation of flies with B. bassiana (Experiment 1)

0.34g of room temperature fungal spores were weighed and placed in a 50mL microfuge tube. These spores were suspended in 25 mL of a 0.03% silwet in deionized water. This fungal suspension was diluted to create the following doses: (undiluted, 10^{-2}, 10^{-3}, 10^{-4}). Adult flies that were 5-7 days old post eclosion were briefly anesthetized with carbon dioxide (CO_2) and measured in a centrifuge tube to 0.5 mL, which corresponds to ~100 flies, or ~50 flies/sex. The measured flies were then spread out on a small petri plate lid that was placed on ice (to continue to anesthetize the flies). Flies from each RIL were sprayed with 7.5 mL of a fungal suspension per dose using a spray tower (calibrations and protocol were followed from Vandenberg 1996). Control flies were only sprayed with the silwet solution without any added fungal spores. After handling, flies were placed into cages and kept at 100% humidity for 24 hours. After 24 hours, the cages were maintained at 25° C at 60-70% humidity with a 12/12-light/dark cycle. Mortality was counted daily and recorded, distinguishing the number of males and females that were dead or lost due to handling. This was done until all of the flies died naturally.

Inoculation of flies with B. bassiana (Experiment 2)

Adult flies that were 5-7 days old post eclosion were briefly anesthetized with carbon dioxide (CO_2) and measured in a centrifuge tube to 0.5 mL, which corresponds to ~100 flies, or ~50 flies/sex. The measured flies were then spread out on a small petri plate lid that was placed on ice (to continue to anesthetize the flies). Flies were sprayed with 5 mL of a fungal suspension (0.03% silwet) containing 10^3 spores/mm^2 of *Beavaria bassiana* (0.034g spores/25mL silwet). These inoculated flies were placed into cages and kept at 100% humidity for 24 hours. After 24 hours, the cages were maintained at 25° C at 60-70% humidity with a 12/12-light/dark cycle. Mortality was counted daily and recorded for ten days, distinguishing the number of males and females that were

dead or lost due to handling. After ten days, the surviving flies were terminated and counted, in order to know the exact number of flies that were in each cage for the experiment.

Fungal Viability Check and Spore Count

To confirm that the fungal spores used in the sprays were viable, a 2mL silwet suspension of a very small amount of spores was sprayed onto a small petri plate containing fungal growth media per inoculation session. The plate was then incubated at 25° C. After 24 hours, the plate was examined for even distribution of spores. After 72-96 hours, the plate was checked for a lawn of fungus growth.

In order to verify dosage sprayed onto the flies, a microscope cover slip was sprayed alongside the flies in each inoculation. The cover slip was then placed in a 50 mL microfuge tube with about 15 small glass beads and covered with 5 mL of 0.03% silwet. A vortex shaker was used to get the spores off the coverslip and into the suspension. The spore suspension was then added to another coverslip over a counting area. Using a disposable pipette, a drop of the suspension was placed onto each of the two grids of two hemocytometers. Using a light microscope, the spores in the four corner squares and center square were counted in a consistent way, as to obtain the best estimation of spores per mm^2.

Data Analysis

R version 3.1.3 (R core team 2015) was used to make all survival plots for the preliminary experiment and also for all data analysis (scripts are in the Appendix). Column charts for the A1 population phenotypes were made in Excel 2013 to depict the survival at day 10 for each RIL.

A two-way Analysis of Variance (ANOVA) was performed to test for variation between RILs and between the three replicates for 22 RILs. The function was as follows:

```
anova<-aov(survival~RIL+replicate+RIL:replicate,data=mydata)
```

Survival curves were generated in R using the ggplot2 data package. The following code was used to generate the graphs:

```
terms<-x
  fmla<-as.formula(paste("Surv(day,censor)~",paste(terms,collapse="+")))
  survdata<-survfit(fmla,data)
with(survdata,{
aa<-data.frame(Condition=rep(names(strata),strata),Time=time,Survival=surv,upper,lower)
aa<-ddply(aa,.(Condition),function (x) { if (min(x$Time)!=0) {
rbind(unique(data.frame(Condition=x$Condition,
Time=0,
Survival=1,
upper=1,
lower=1))
,x)}  else x
})
bb<-colsplit(aa$Condition,",",terms)
bb<-mapply(function (x) gsub("^.*=","",x),bb)
cc<-data.frame(bb,aa[,-1])
cc<-within(cc,{
upper<-ifelse(Survival==0&is.na(upper),0,upper)
lower<-ifelse(Survival==0&is.na(lower),0,lower)
})
return(cc)})}
```

QTL mapping analysis was done twice, once on 22 RILs that were three-fold replicated while averaging survival over the three replicates, and once on 125 RILs, including ones that were not yet replicated. Percent survival data was input to an Excel worksheet containing only two columns of information: (1) the RIL ID of each line (obtained from http://wfitch.bio.uci.edu/~dspr/riltable/index.html), which was labeled “patRIL” and (2) the average proportion survived at day 10 for each RIL, which was labeled “survival.”

The DSPR QTL data packages were authored and maintained by Elizabeth King of the University of California Irvine. The DSPRqtl analysis package was downloaded and installed into R (on a computer with internet connectivity) with the command:

install.packages("DSPRqtl", repos = "http://wfitch.bio.uci.edu/R/", type = "source")

The DSPRqtlDataA, and the DSPRqtlDataB packages were downloaded from:

http://wfitch.bio.uci.edu/R/src/contrib/DSPRqtlDataA_2.0-1.tar.gz

http://wfitch.bio.uci.edu/R/src/contrib/DSPRqtlDataB_2.0-1.tar.gz

and installed using the commands:

install.packages("DSPRqtlDataA_2.0-1.tar.gz", repos = NULL, type = "source")

install.packages("DSPRqtlDataB_2.0-1.tar.gz", repos = NULL, type = "source")

The phenotype data was read into R and a genome scan was performed to determine LOD scores at each locus in the genome. The scan was performed by sampling 1000 positions at a time. The DSPRscan was performed using the following code:

scan <- DSPRscan(survival ~1, design = "inbredA", phenotype.dat = mydata, id.col='patRIL')

A permutation test was also performed to evaluate the LODdrop value that should be used for the actual analysis of QTL peaks. The permutation test was as follows:

perm <- DSPRperm(survival ~ 1, design = "inbredA", phenotype.dat = mydata, id.col='patRIL', alpha = 0.01)

Using the output from the genome scan, peaks were determined for loci with an LOD score above 6.8. The following function generated the peaks:

peaks <- DSPRpeaks(scan, method = 'both', threshold = 6.8, LODdrop = 11)

The output from the scan was used to generate a visualization of the significant peaks:

DSPRplot(list(scan), threshold=6.8)

The QTL analysis was performed using a 0.99 BCI (alpha = 0.01) and both BCIs and LOD drop intervals were calculated. The BCI probability is the Bayesian Credible Interval probability, which is the desired nominal Bayes fraction for support intervals. With this credibility level, the analysis produces confidence intervals for each locus. Once loci were identified, these genomic positions were referenced through the Flybase Genome Browser in order to establish genes containing the loci (http://flybase.org/cgi-bin/gbrowse2/dmel/). Genes were said to contain a locus if there was an open reading frame for a protein-coding gene contained within 5kb for the locus. (a list of BCI values and LOD scores for each locus can be found in the appendix).

Results:

I. Results from Experiment 1

Determination of Optimal Dose and Quantitative Measure for Resistance

Initially, I needed to determine two aspects of my experiment before expanding its scale: (1) what dose of fungus would be optimal for quantifying resistance, and (2) what phenotypic measure would be best for estimating resistance to the fungal pathogen (both in terms of analysis and methodological practicality).

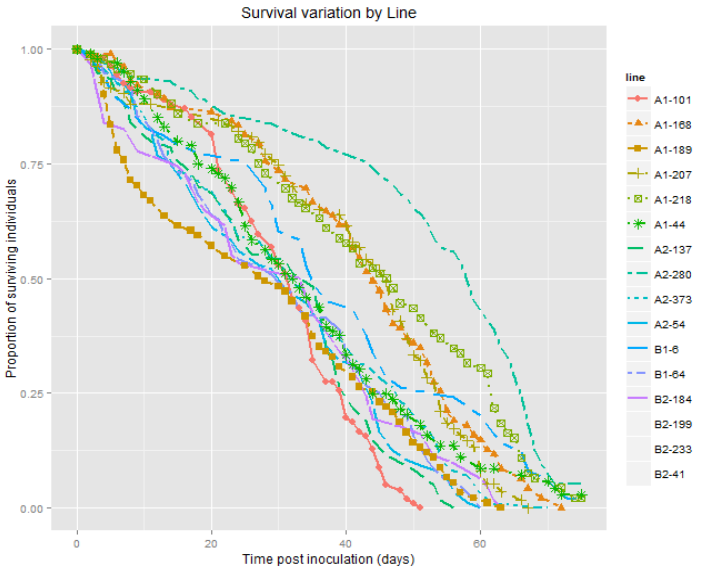
In order to determine a dose of fungus inoculation that allowed for high enough mortality for an observable resistance phenotype, I first inoculated 18 RILs with four different doses of *B. bassiana.* I suspended 0.34 grams of *B. bassiana* strain ARSEF 12460 in 25 mL of 0.03% silwet in DI water and then did serial dilutions. In units of spores/ mm^2, the doses I used were: 7.23, 47.52, 303.72, and 75671.49. Flies in the control group were handled identically but not exposed to fungal spores.

In the control group I saw a fair level of natural genetic diversity in survival among the RILs (Fig. 1a), and the survival curves looked akin to those of other Drosophila populations, suggesting that the RILs are robust and healthy. With the lowest inoculation dose, survival did not deviate much from that of the controls; therefore, I determined that this dose would be too weak to use in further experiments. Though no statistics were performed, it seemed that the lowest dose did not affect survival that much and was smaller than expected. For flies inoculated with the second lowest dose, the effects of infection on survival become clear (Fig. 1c). By day 20, more than half of the flies were dead for most of the RILs, whereas the control only exhibited at most 25% death by that time. Survival curves for flies inoculated with the two highest doses looked very similar to one another, despite the two orders of magnitude difference between these high doses. This result suggested that after some fungus dose, increasing concentrations of fungus would have little impact on mortality. At

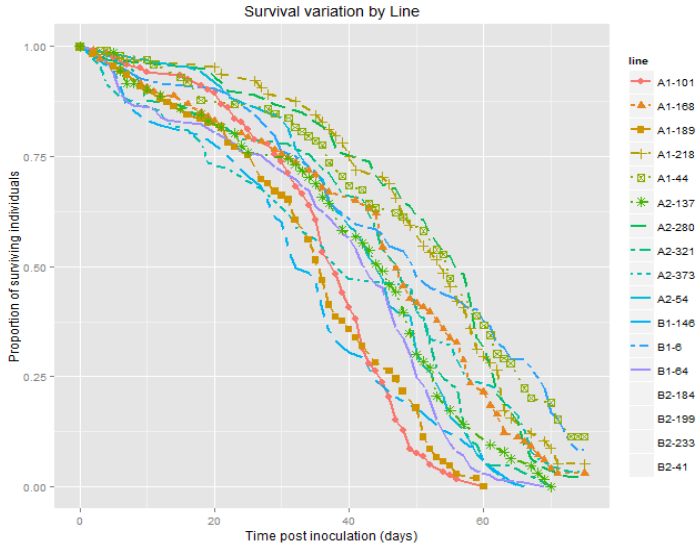
both of these high doses, there was substantial variation in survival after inoculation among the RILs. For future experiments I decided to use a dose that was intermediate to these two, aiming for 10^3 spores/mm^2.

In deciding what quantitative phenotypic measure to use for estimating resistance to *B. bassiana* in future experiments, I tested truncation of the survival curves at various days after inoculation to determine the variation in proportion of flies that survive to that age. Truncating the data at day 10 seemed ideal because on day 10 there were negligible differences in survival among the RILs in the control group (Fig. 2a), but large differences in survival among the RILs in the inoculated groups (Fig.2c-e). I decided that the best phenotypic measure would be proportion of surviving flies alive at day 10 of the experiment (% survival), as opposed to slope of the survival curve, time to 50% death, or time to 100% death. Using a short 10-day window also allowed me to test many more lines in rapid succession, giving me the opportunity to measure the resistance of hundreds of RILS with multiple replicates within a reasonable time frame.

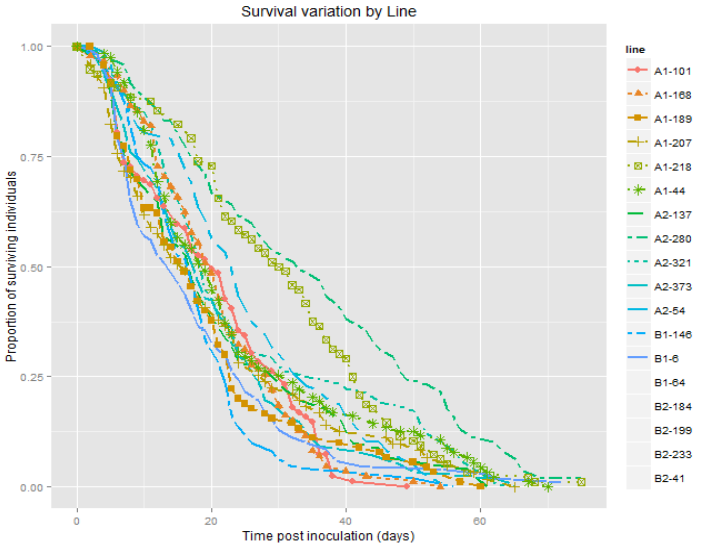
A.



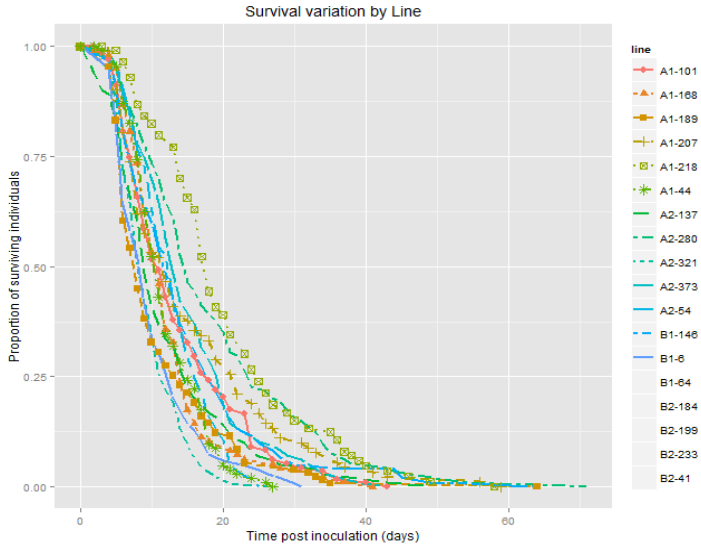
B.



C.



D.



E.

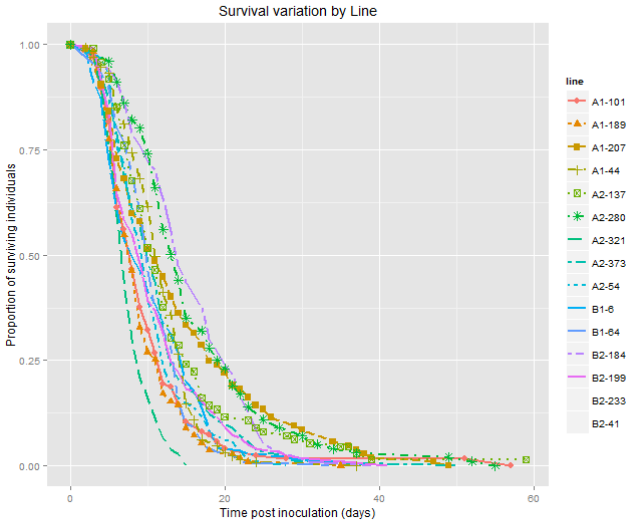
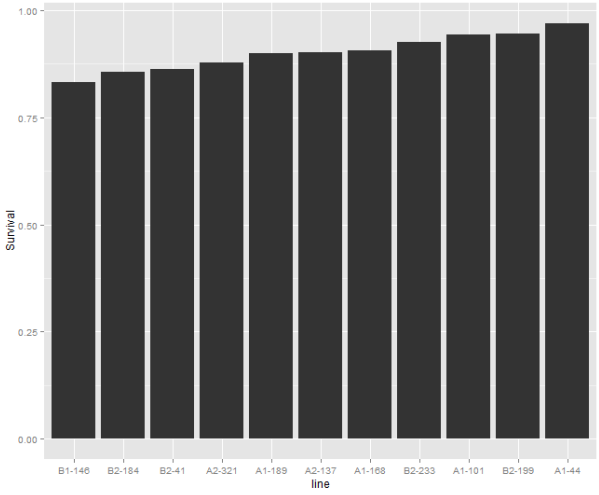
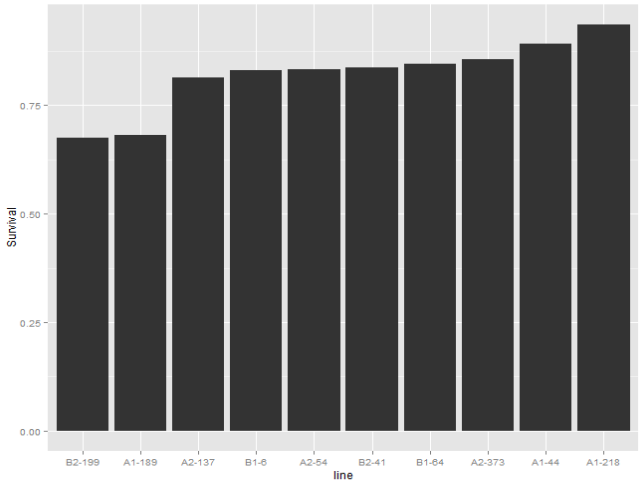


Figure 1. Survival of RILs from Experiment 1. The graphs show survival curves for RILs sprayed with silwet control (A), 7.23 spores/mm2 (B), 47.52 spores/mm2 (C), 303.72 spores/mm2 (D), and 75671.49 spores/mm2 (E). Each color curve represents a different RIL. Higher doses showed greater mortality early on post infection than lower doses. Graphs show great variation in RIL response to infection.

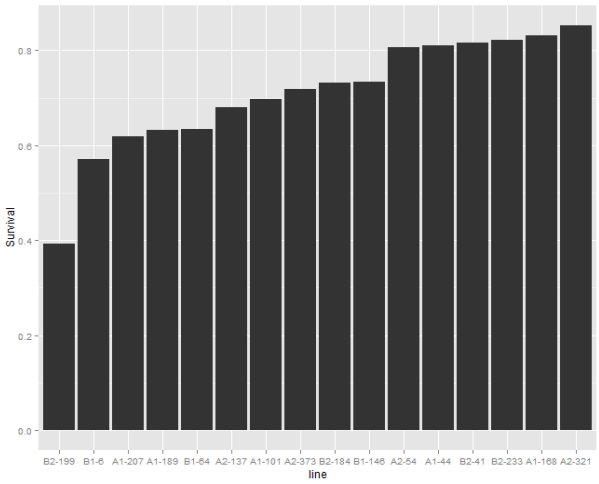
A.



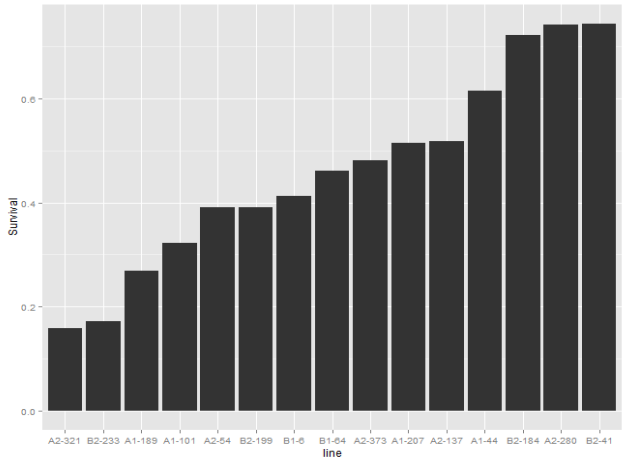
B.



C.



D.



E.

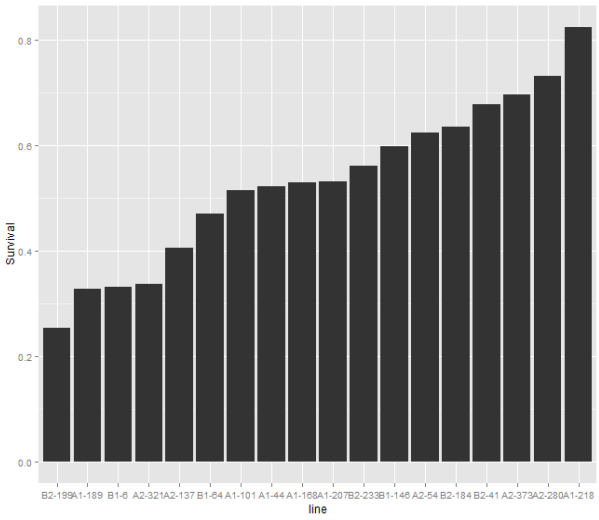


Figure 2. Proportion of individuals surviving after 10 days from Experiment 1. The bars show percent survival for each RIL 10 days after infection for RILs sprayed with silwet control (A), 7.23 spores/mm2 (B), 47.52 spores/mm2 (C), 303.72 spores/mm2 (D), and 75671.49 spores/mm2 (E).

II. Results from Experiment 2

The RILs used in the main experiments were all from population A1 of the DSPR. To date, only one replicate of 96 RILs, two replicates of 7 RILs, and three replicates of 22 RILs have been phenotyped for resistance to *B. bassiana*. Figure 3 shows the percent of flies surviving by day 10 for all of these RILs, regardless of replicate number. The bars for the RILs with multiple replicates are actually averages of survival across replicates. It illustrates the great diversity in resistance among the RILs, even within the A1 founder population. This means that QTL analysis can be used to map key loci in the genome that contribute to resistance against fungal infection.

RILs show consistent phenotypes across replicates

Only 22 of the 125 RILs were phenotyped three times (same RILs tested multiple times), which is a comfortable level of replication. These 22 RILs seem to consistently show the same or similar quantitative resistance phenotype (survival) over multiple replicates (Figure 4). For the most part, there tends to be very little variation between replicates, as demonstrated by the small error bars. A two-way ANOVA test was performed to statistically test for the variation between replicates for each RIL. There was a significant difference between RILs ($p=3.79*10^{-3}$), but there was no significant difference between replicates of each RIL ($p=0.89$). Therefore, there was little variation across replicates, indicating that the environment did not contribute much to the resistance phenotypes. Thus, the diversity in survival between RILs is probably linked to their genetic diversity.

Based on Figure 4, is seems clear that either the experimental procedure has been consistent and that the environment (humidity, temperature, handling) has been kept the same for each round of infection and phenotyping, or that the RILs are so robust that variation in environmental conditions do not matter. Further, based on survival for the 22 RILs with triplicate (Figure 4), it can be inferred

that variation in survival for all RILs is due to genetic diversity, since survival does not change much over each replicate for every RIL.

Variation in survival between RILs still exists when accounting for sex (Figure 5). This data was collected based on the 22 RILs with three replicates and the 7 RILs with two replicates. It is also apparent that female response to infection differed much from that of males. For 22 of the 29 RILs, females tended to show lower survival than males, suggesting that a sexual dimorphism exists in *D. melanogaster* when it comes to resistance against infection by a fungal pathogen.

QTL analysis shows importance of loci in chromosome 2L and 3R

With the initial library of survival phenotypes, a QTL analysis was performed on 2 sets of data: (1) survival phenotypes for all lines regardless of whether they had been replicated 1, 2, or 3 times, and (2) survival phenotypes for only the 22 RILs with three replicates. For (1), only 1 locus in chromosome 2L was found, whereas (2) showed 16 loci in chromosome 2L and 3R. The significant loci can be visualized as peaks at different locations on the chromosome (Figure 6). Peaks are defined as significant if the QTL has an LOD score of 6.8 or greater. These loci revealed many genes that have different biological function. However, the genes can be classified into protein coding genes involved with the following features: (a) gene regulation at the transcriptional, translational, and protein level; (b) immunity and damage repair; (c) metabolism, homeostasis, or development; and (d) immunoglobulin proteins with little to no known function. For the time being, I will not analyze transposable elements or nc-RNAs within the peaks given in the outputs from the QTL analysis. That will be an area that will explore at a later date when I have phenotyped more lines and can execute more specific and detailed QTL analyses that account for replicate variance, sex, and other experimental conditions. Table 1 contains a table with a summary of the quantitative trait loci in the genome, along with the genes contained within the peak confidence intervals and their known functions.

The QTL analysis identifies known genes directly involved in the immune response

The QTL associated with peak [6] is within the gene hml, which encodes Hemolectin, a protein involved in clotting the hemolymph of the fly in order to initiate wound healing at the site of puncture on the exoskeleton (Scherfer et al., 2004). Clotting serves as an important immune defense through the formation of a barrier against infection. Most strikingly, the QTL analysis also recognized peak [4], which contains the gene for the protein Membrin. Membrin is one of 184 proteins essential for efficient phagocytosis in *D. melanogaster* (Stroschein-Stevenson et al., 2005). This role was tested against *Candida albicans*, a major fungal pathogen for humans.

A third gene, ird1, was found to impact resistance. Ird1 is activated during infection and induces the expression of NF-kB, a transcription factor which activates the expression of immune factors. Ird1 thus positively regulates the expression of antimicrobial peptides (AMPs) during infection. AMPs are humoral effectors of the innate immune response that are toxic to parasites (Ganz, 2003).

Genes involved in the regulation of expression may affect resistance

The genes from peaks [1] and [4] code for Ubiquitin specific protease 10 (USP 10) and Ubiquitin-conjugating enzyme variant 1A (Uev1A), respectively. USP 10 is a homolog in humans and was actually discovered in flies through sequence identity (D'Andrea and Pellman, 1999; FlyBase Curators 2008). USP 10's function is to cleave ubiquitin from proteins. In this way, it helps to positively regulate Notch signaling, which is important for development (Zhang et al. 2012). Uev1A is involved in polyubiquitination of proteins, such as DIAP1. DIAP1 is a protein which, when ubiquitinated, is activated and suppresses apoptosis in *Drosophila* (Herman-Bachinsky et al., 2006). Uev1A also serves as an important ubiquitin ligase required for early embryonic development (Merkle et al., 2009). UevA1 has a role in the IMD innate immune pathway as well, which will be explored further in the discussion.

The genes from peaks [6], [10], and [12-16] produce transcripts for proteins involved in binding DNA or RNA in terms of regulating transcription and translation. Peak [11] represents a gene involved in the demethylation of lysine residues in histones (Lagarou et al., 2008). In sum, these proteins, along with the USP 10 and Uev1A are involved in the regulation of genes, cell division, and cell life. Their significance in the QTL analysis suggests that these genes may regulate cistrons directly involved in resistance against fungal infection. However, it still remains unclear exactly which regulatory pathways would induce a higher or lower survival phenotype.

QTL recognizes certain genes involved in metabolism and homeostasis

QTL [2], [3], and [9] contain genes involved in metabolism and transport (Table 1). The transport proteins include a glucose transporter, which is homologous to a human transporter. Cytochrome c1 is also a gene that is conserved and very essential in oxidative phosphorylation for both flies and humans. CHMP2B is an endosomal protein that is involved in transport. Endosomes can be used to transport materials to lysosomes, which are involved in the destruction of phagocytosed parasites (Mayorga et al., 1996; Allen & Aderem 2002). Klp64D is a kinesin involved in axon transport along microtubules and the development of wings (Vuong et al., 2014). It is therefore a key gene for motor function and sensory perception. Lama currently only has known functions involving epithelial tube formation and imaginal disc cell regulation (Peters et al., 2013; Klebes et al., 2005).

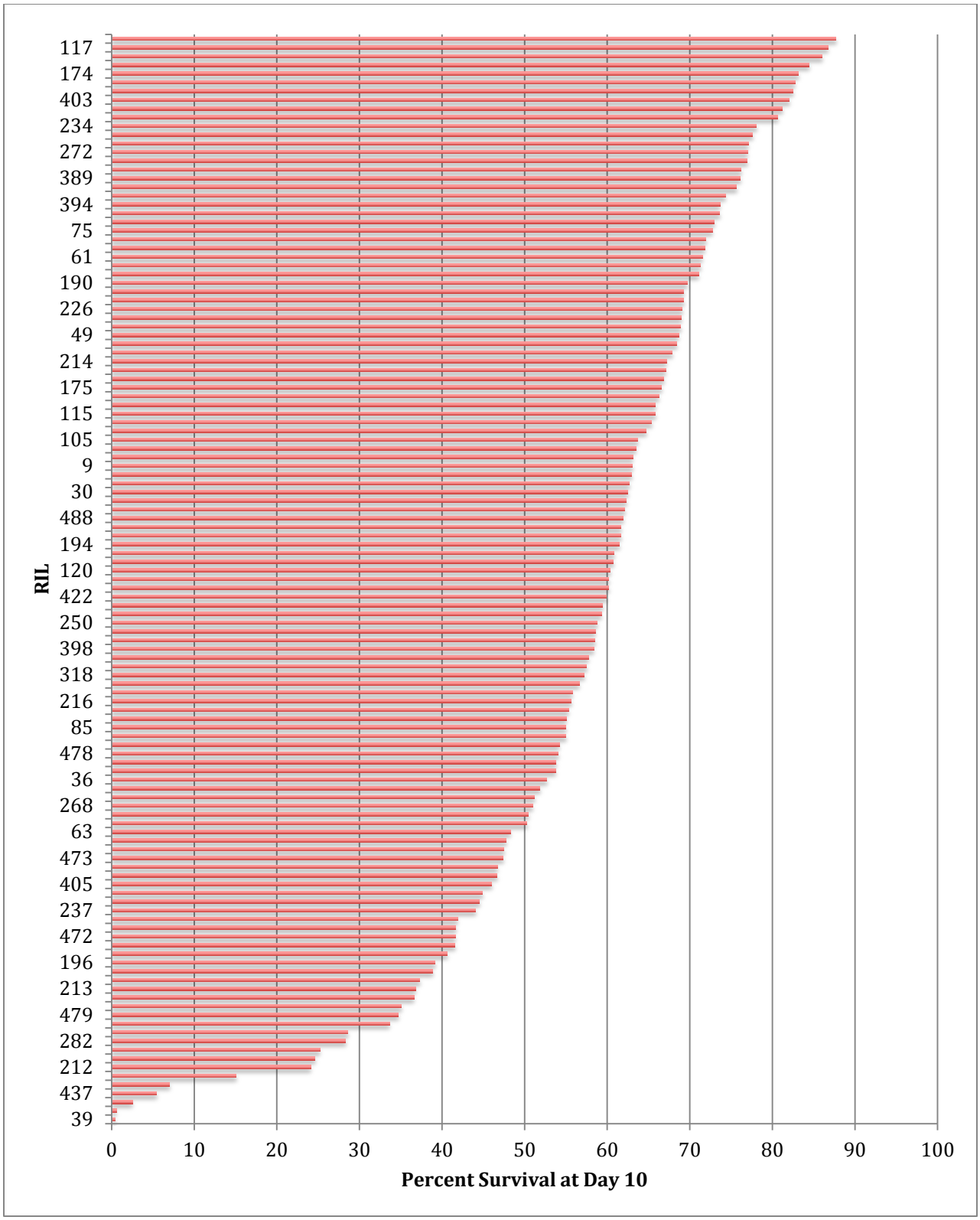
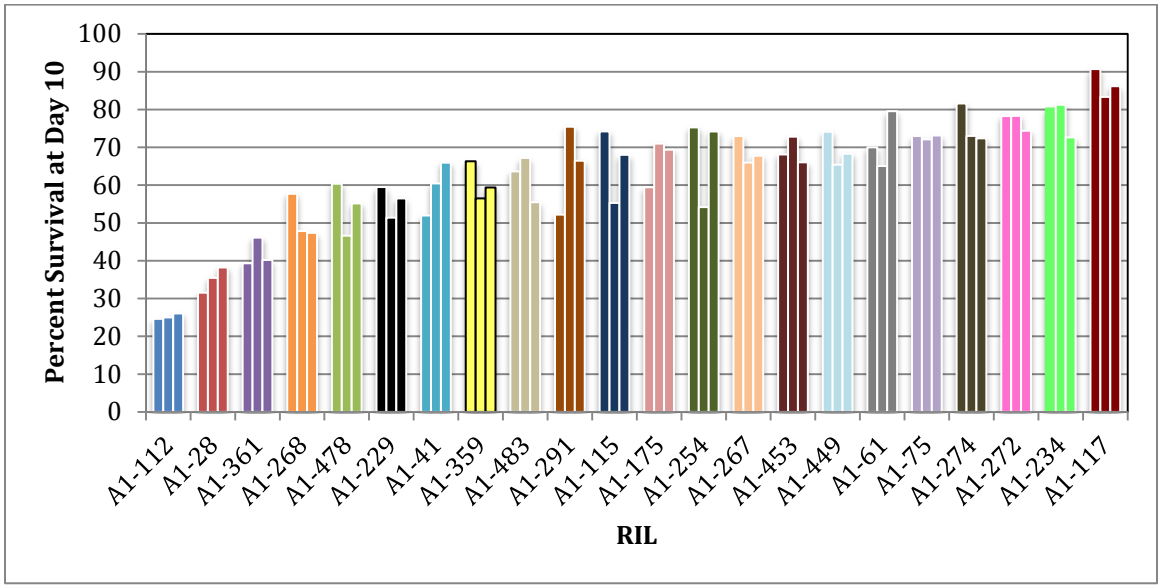


Figure 3. Percent of individuals surviving at day 10 from Experiment 2. The survival for each of the 125 RILs at day 10 was plotted from least to greatest. Values for the RILs with more than one replicate were averaged over the replicates to obtain one value per RIL.

A.



B.

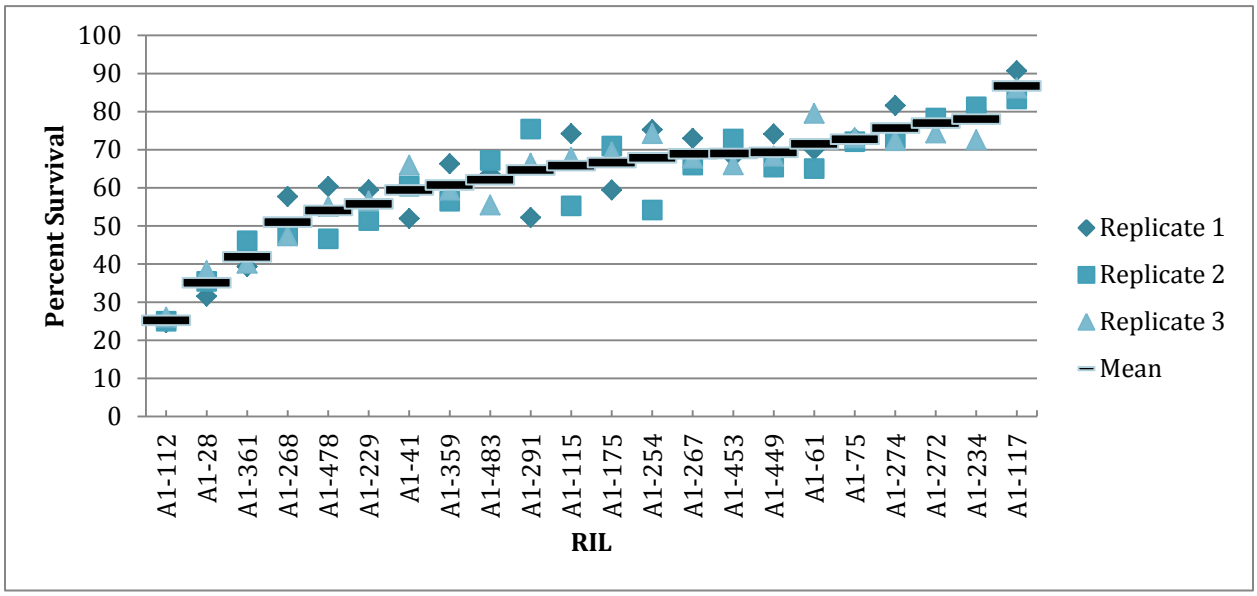
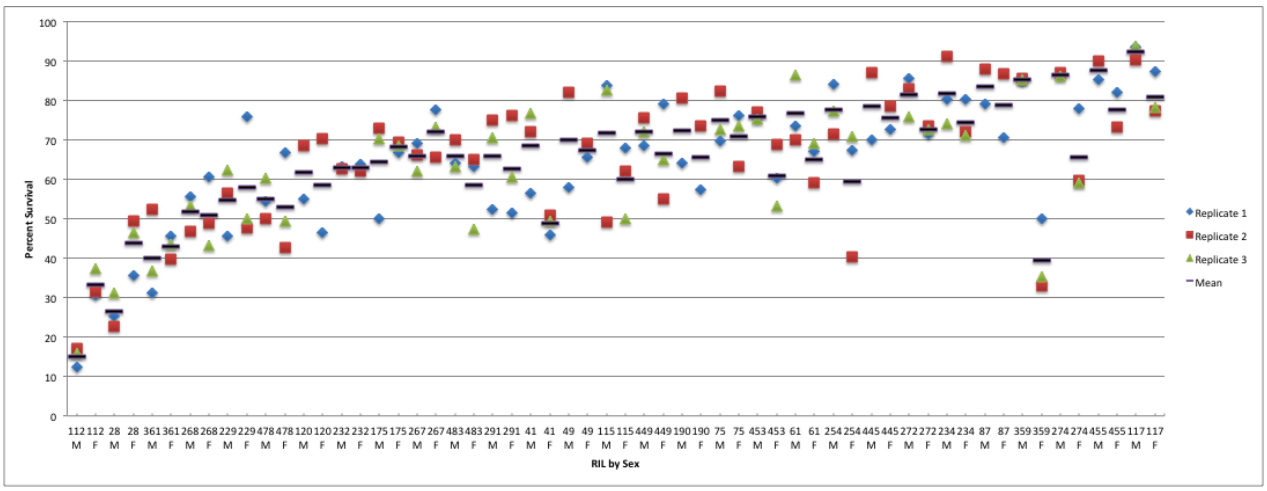


Figure 4. Percent survival at day 10 for 22 RILs. (A) Shows the survival for each of the three replicates for each RIL that was tested in triplicate. (B) Shows the mean survival over the three replicates for each RIL as well as individual replicate values.

A.



B.

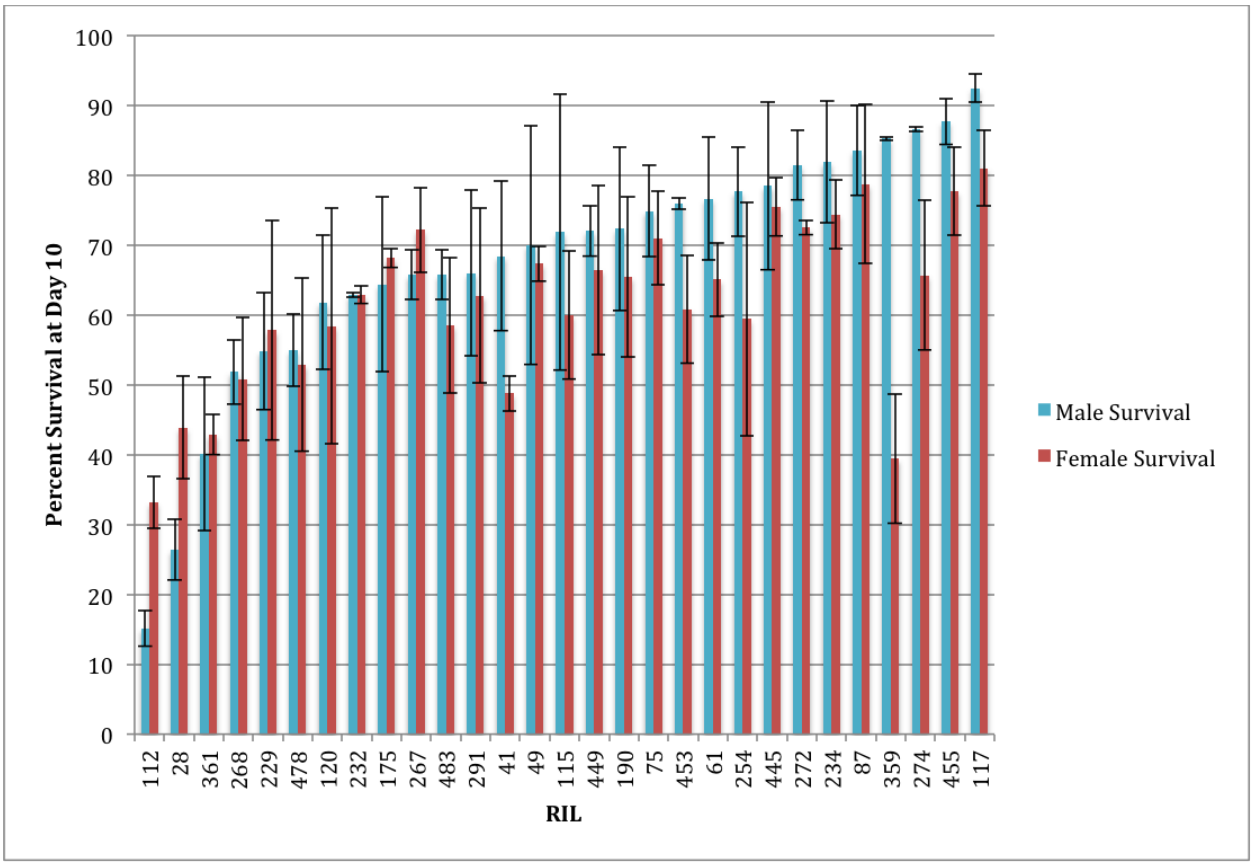
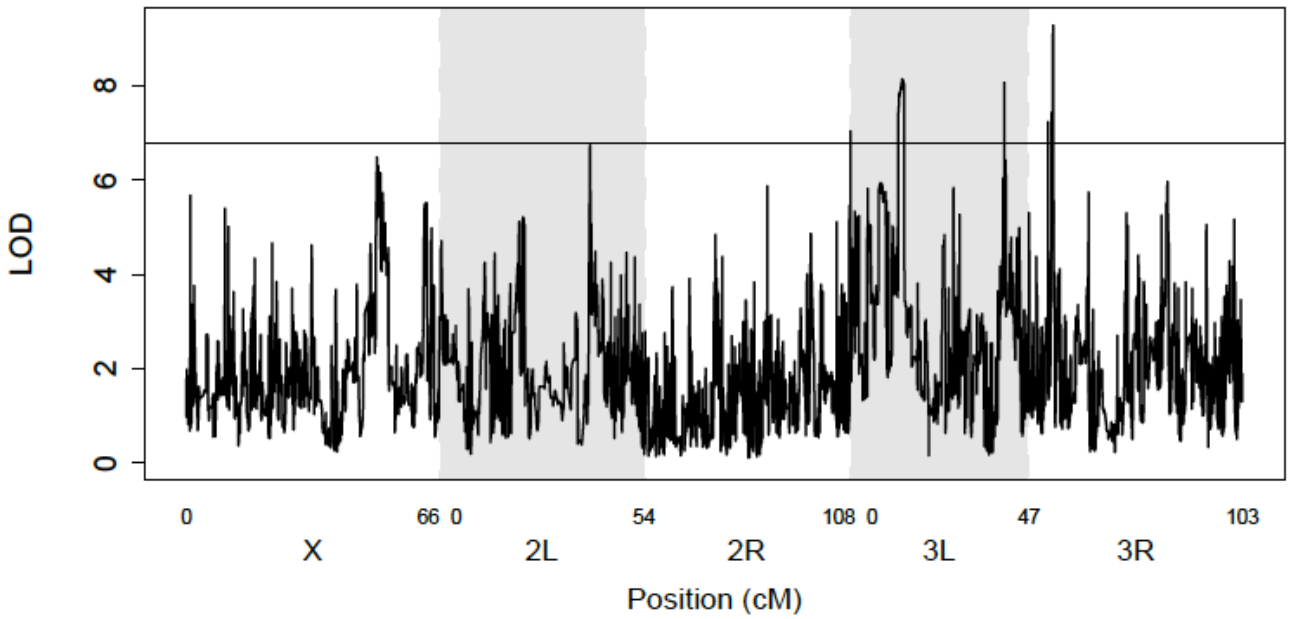


Figure 5. Percent survival at day 10 per sex for each RIL. Graphs show survival per sex of the 22 RILs with three replicates and 7 RILs with two replicates. (A) Shows survival per sex per RIL over for each replicate, with mean values per sex given. (B) Demonstrates averages of survival (with standard deviations) over the two or three replicates for males and females for each of the 29 RILs that were tested with more than one replicate. Data indicates a difference in sex response during infection.

A.



B.

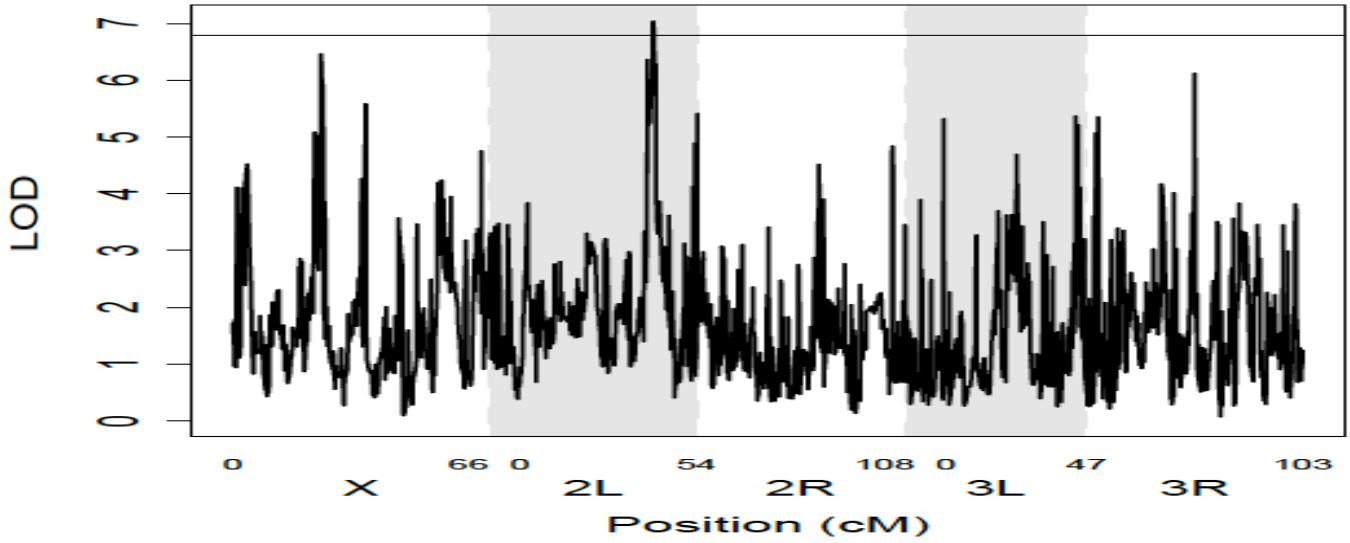


Figure 6. QTL peaks throughout the *D. melanogaster* genome as given by the genome scan. The background shading (white/grey) represents the major chromosome arms. (A) Shows the peaks from an analysis using only the 22 RILs with three replicates. (B) Shows the peaks from a genome scan using all 125 RILs tested (regardless of replication number).

Table 1. Summary of QTL. Peaks 1-17 correspond to the genome scans performed using only the 22 RILs that had three replicates. Peak [18] was identified using a genome scan using all 125 RILs tested. Letters in "gene function" correspond to classification of function under the following categories: (a) gene regulation, (b) immunity,
(c) metabolism/homeostasis/development, (d) immunoglobulin.

Peak	Locus	LOD Score	Gene(s)	Protein(s)	Gene Function(s)	Actual Position of gene(s)	Sources for function
1	3L: 880000	7.033	Usp10	Ubiquitin specific protease 10	Deubiquitination of proteins, positive regulation of notch signaling (a)	3L:871,899..895,313	D'Andrea and Pellman, 1999; Zhang et al., 2012
2	3L: 5120000	7.846	CHMP2B	Charged multivesicular body protein 2b	endosomal/vacuolar transport (b,c)	3L:5,139,619..5,140,473	Capalbo et al. 2012
3	3L: 5350000	8.077	lama	lamina ancestor	dorsal appendage formation; imaginal disc development (c)	3L:5,343,619..5,355,923	Peters et al., 2013; Klebes et al., 2005
			Klp64D	Kinesin-like protein at 64D	Kinesin, ATP binding, regulation of senses (c)	3L:5,356,864..5,359,392	Jana et al., 2011; Sarpal et al. 2003
			cyt-c1	Cytochrome c1	oxidative phosphorylation (c)	3L:5,360,211..5,362,798	FlyBase 1992
4	3L: 5370000	8.141	Membrin	Membrin	SNARE involved in vesicle transport, gene is essential for the phagocytosis of fungal parasites (b)	3L:5,366,455..5,367,381	Stroschein-Stevenson et al., 2005
			Uev1A	Ubiquitin-conjugating enzyme variant 1A	polyubiquitination of proteins in order to regulate transcriptional activation of genes in suppression of apoptosis, regulation of development, part of IMD pathway protein cascade (a,b)	3L:5,362,884..5,366,225	Herman-Bachinsky et al. 2007; Merkle et al. 2009; Zhou et al. 2005
5	3L: 5560000	8.048	--	--	--	--	--
6	3L: 13700000	8.065	Hml	Hemolectin	Coagulation/chitin binding (b)	3L:13,846,054..13,860,001	Scherfer et al., 2004
			bru-3	Bruno-3	mRNA binding and negative regulation of translation (a)	3L:13,521,907..13,803,400	Lasko 2000; Delaunay et

							al., 2004
7	3R: 8370000	7.229	CG45263	No Name (Immunoglobulin subtype 2)	Unknown (d)	3R:8,364,328..8,477,617	--
8	3R: 8970000	7.429	--	--	--	--	--
9	3R: 8990000	6.825	CG31100	Similar to Facilitated glucose transporter member 6 (humans)	Glucose transporter (c)	3R:8,995,229..9,003,650	Flybase, 1992
10	3R: 9010000	6.926	tgo	Tango	contributes to RNA polymerase II distal enhancer sequence-specific DNA binding transcription factor activity, myosin binding (a)	3R:9,016,773..9,020,022	Kozu et al. 2006
11	3R: 9050000	6.806	Kdm2	Lysine (K)-specific demethylase 2	histone H2A ubiquitination and histone H3-K36 demethylation (a)	3R:9,052,495..9,063,245	Lagarou et al., 2008
12	3R: 9100000	7.280	pum	Pumilio	mRNA binding, regulation of translation, important for neuronal development and maintenance (a)	3R:9,066,343..9,237,682	Deng and Lin, 2001; Menon et al., 2004
13	3R: 9170000	7.720	pum				
14	3R: 9190000	8.550	pum				
15	3R: 9210000	8.266	pum				
16	3R: 9230000	9.279	ird1	Immune response deficient 1	regulation of AMP expression and activator of NF-kB (induced by infection and starvation) (b)	3R:9,240,757..9,245,869	Wu et al. 2007
17	3R: 9280000	6.966	Rt1a{}1276	--	transposon	--	--
[18]	2L: 10900000	7.041	Dpr2	Defective proboscis extension response 2 (immunoglobulin subtype 2)	Sensory perception of chemical stimulus (c,d)	2L:10,917,015..10,964,153	Nakamura et al., 2002

Discussion:

Traditional QTL analyses are able to identify loci with a resolution of about 20-30 cM (Kearsey, 1998). Although centimorgan (cM) refers to a genetic distance (based on recombination frequency between two points) and not a physical distance necessarily, it can be estimated that 1cM is about 500kb (Zhai et al., 2003). Therefore, classical QTL tests have a confidence interval of about 10-15Mb. Although tests can identify a single locus (nucleotide) in the genome that affects a phenotype, the true locus can be anywhere in the confidence interval. The DSPR is capable of increasing this resolution by an order of magnitude, to 1.5 cM or 750kb (King et al., 2012). However, this resolution is achieved by using more than 800 RILs. Up to date, I only have resistance data for 125 RILs, and only 22 of those were three-fold replicated so far. Therefore, there are two factors that limit the power and resolution of my QTL analysis: the amount of RILs being tested, and the level of replication for each RIL.

I first tested only 22 RILs, from which I gained the first 17 peaks, and then I tested all 125 RILs, from which I got the 18^{th} peak. Though I did have QTL, most of the QTL had high confidence intervals (Appendix). This means that the resolution of QTL was not high. In order to identify the genes containing each QTL, I restricted my search to within 5kb of each QTL since I would identify too many genes if I search within the intervals. This 5kb number was arbitrary.

Unless the QTL effect is large and environmental variation is greatly reduced by replication, it is difficult to reduce the confidence interval (Kearsey, 1998). In the first experiment of 22 RILs, the replicates were sprayed at the same time and phenotyped simultaneously. That means that each of the three replicates were subjected to very similar environmental conditions and handling. Since there was little variation between replicates as indicated by the ANOVA test, then the variation between different RILs had to come mostly from a genetic variation. However, it is impractical to spray all 300 RILs at the same time in this manner, so some RILs need to be assayed at different times from others, which means that there would be environmental variation between the assays.

Based on previous experiments, I expected that the phenotype would be sensitive to environmental changes, in particular to the humidity and temperature immediately after inoculation. Since these environmental factors are hard to control in the laboratory, especially given the seasonal changes in lab humidity, I chose to sample 3 replicates for each of the 300 RILs. These replicates have been sprayed independently and at different times from one another. Using this replication, I will have a more precise survival estimate and therefore should also have more accuracy in the QTL mapping. For this reason, I plan to test 3 replicates for 300 RILs, instead of using just 1 replicate of 900 RILs, for example, which would be a better approach if the phenotype were less environmentally sensitive.

Due to the low resolution of my QTL, it is possible that I have false positives among the candidate genes that I identified. Two of my 18 QTL contained no protein coding genes, and 1 of them contained a transposon. Assuming that the transposon may not have an effect, my minimum false positive rate is 3/18 = 0.167. This also assumes that none of the other 15 QTL with protein coding genes are false positives, though they may be. As to which of the 15 other loci are false positives cannot be calculated or inferred at this time. The only way to be sure is to perform molecular genetics experiments. These experiments would involve taking a background with some easily identifiable phenotype or balancer phenotype, followed by making a copy of that background with a knockout of the candidate gene of interest. Both the background controls and the mutants would be sprayed with fungus and survival for both groups would be determined at day 10. Then both groups can be compared using statistics (an ANOVA or t-test) to see if there is a significant difference in survival. If there is, then the candidate gene now becomes a gene that is involved in affecting the immunity phenotype. If not, then that candidate gene/locus can be said to have been a false positive.

Ultimately, the genes that I have identified through the DSPR genome scan are only candidate genes that may have an effect on surviving fungal infection. I nevertheless provide previous evidence that suggests ways in which these genes might be involved in regulating immunity

in *Drosophila*, also stating which genes share homologs with humans. Based on the literature, it seems that the genes could be involved in surviving infection. I also give speculation of alternate functions that may play a direct role in the immune response. The impact of my research is to provide a starting point for identifying novel genes involved in resistance against fungal infection. It helps to reduce the ~17,000 genes (minus the ones we already know are involved in immunity) to a handful of genes which can then be tested.

Even with just three replicates of 22 RILs, the QTL analysis was able to find many of genes that are possibly involved in immune defense. The QTL analysis defined three candidate genes that have been previously shown to play a direct role in immune defense, such as Hml and Membrin. Further, Membrin was found to have an active role in cellular immunity against fungi in particular. With such accurate tracing of known genes involved in resistance, there exists much promise that the rest of the genes that were found could have something to do with survival during infection.

Uev1A is also an essential gene in the IMD immune pathway in Drosophila. Experiments have shown that Uev1A is required to activate Tak1, which activates IKK, which activates the NF-kB, Relish (Zhou et al., 2005). As previously mentioned, NF-kB is a transcription factor which activates the transcription of AMPs. Since Uev1A has a direct role in a branch of the immune response, it has an effect on resistance, which is maybe why it appeared as one of the QTL. UevA1 also uses NF-kB activation to inhibit apoptosis (Syed et al., 2006). The great array of homeostatic pathways that Uev1A, including immunity, suggests that other candidate genes evaluated through the QTL analysis could also be involved in immunity even if research has only shown them to have other roles.

Hemolectin is essential in coagulation at the site exoskeletal damage. That this gene may be important in resistance against fungus makes sense. We know that *B. bassiana* infects its fly host by physically piercing the cuticle, thereby causing damage. Flies with more efficient or higher expression of hemolectin may be better able to contain the fungal infection early on, thus delaying

the course of pathogenesis. But hemolectin is also known to bind chitin, which is a constituent of the fly exoskeleton. But chitin is also a major component of the cell wall of fungi. It is also likely that hemolectin has a secondary effect, where it binds fungus and prevents its growth.

Membrin is a SNARE protein that mediates vesicle fusion. It is used to help transport vesicles from the Endoplasmic Reticulum to the Golgi Apparatus (Ossipov et al., 1999). However, investigations suggest that Membrin is also involved in vesicle transport during phagocytosis (Stroschein-Stevenson et al., 2005). This implies that Membrin may have an alternate function in helping to induce phagocytosis or maturation of the endosome. Nevertheless, what is known for sure is that it is a protein required for phagocytosis. I speculate that flies expressing more Membrin or having certain membrin alleles may be able to phagocytose the fungal cells quicker and more efficiently. This allows the phagocytes to kill more fungal cells over time, which can make all of the difference early on in infection. Humoral immunity also coordinates with cellular immunity in the innate immune response, and the production of AMPs is a key feature of humoral immunity in Drosophila. Ird1 is activated during infection and induces the production of AMPs. There may be RILs with alleles of Ird1which induce greater production of AMPs.

But when thinking of the effectiveness of an immune response, it is not enough to simply think of the genes that directly interact with the fungus. Ultimately, the immunity genes that I have explained above need to be tightly regulated, especially since they are only expressed during infection or stress (except Membrin). The regulatory genes or sequences, which interact with metabolism and immunity, need to be considered. In light of this, it is reasonable that the QTL analysis found genes that encode regulatory proteins involved in modulating transcription, translation, and protein half life. My hypothesis is that these control factors are tailored in more resistance RILs in such a way that immunity genes such as Ird1 are upregulated and expressed in higher numbers than in other RILs.

I found a diverse group of regulatory proteins that are possibly involved in immunity. These include regulation of translation via mRNA binding (Bruno-3 and Pumilo), regulation of transcription through recruitment of RNAPII (Tango), demethylation of histones which can increase or decrease transcription (Kdm2), and control of transcription by ubiquitination or deubiquitination of proteins (Uev1A and USP 10, respectively). But the most striking observation that can be made is that relationships between these different proteins listed here, which I will now elaborate on.

Ubiquitination labels proteins to a certain fate, usually recognition by a protease, followed by degradation. However, ubiquitination of certain proteins can label them to be acted upon by proteins other than proteases. For example, the ubiquitization of histones can cause them to interact with modifying proteins, such as acetylases. Previous research has shown that Kdm2 cross talks with ubiquitination proteins. During gene silencing, histone H2A monoubiquitination is coupled to the removal of a methyl group of Lys 36 in H3 (Lagarou et al., 2008). It is possible that Uev1A can mediate ubiquitination of H2 and Kdm2 may assist in the demethylation, meaning that Uev1A could interact with Kdm2 in order to regulate genes. However, this has not been tested and is merely a hypothesis at the moment. However, the QTL has revealed possible alternate functions and relations of known genes that can be confirmed or denied through experiment.

The role of USP10 in resistance is not clear, but it does have a role in deubiquitination and Notch signaling. My hypothesis is that Notch signaling may interact with immune signaling in some way that is not currently understood. Likewise, Pumilo is another regulatory protein that is currently known to be involved in neural signaling. These genes may provide clues about links between neural pathways and immunity. Further, perhaps USP10 can counter the silencing of genes through deubiquitination of histones or other regulatory proteins. Bruno-3 downregulates translation of transcripts, so it is possible that Bruno-3 interacts with genes that are antagonistic to immunity, which can include insulin synthesis or other metabolic processes.

Metabolism and immunity are intrinsically linked (Weiss et al., 1995). We currently know that changes in diet (and therefore, metabolism) can affect *D. melanogaster's* ability to survive infection (DiAngelo et al., 2009). Further, many pathways involved in metabolism, such as insulin signaling, are involved in immune signaling. Thus, it would be very reasonable for some of the genes from a QTL analysis of resistance phenotypes to include metabolism and housekeeping genes. In my experiment, three genes were found which we currently know have a role in metabolism. Just as important, these genes have homologs in human beings, meaning that the relationships between homeostatic pathways and immunity in flies can be directly determined in humans, since the genes and pathways are shared. Though humans and flies do not share the same physiology, they may share similar biochemistry. So although humans have a more complex nervous system from flies or a more complex metabolism, the most basic constituents of each these systems, which are shared in flies, can still interact with the basic innate immune response.

The glucose transporter may serve a role in shuttling needed resources to cells involved in the immune response, or to sequester circulatory sugars from the pathogen. Cytochrome c1 is directly linked to energy production for the fly. RILs with better cytochromes may get more energy in the form of ATP per glucose or monosaccharide. This means that given the same resources, a RIL with better sugar transporters and better cytochromes can process more energy and at a faster pace. This means that there are more resources to allocate towards surviving an infection. Klp64D is a kinesin designed to transport macromolecules throughout the cell, which may further contribute to efficient mobilization against infection.

CHMP2B is a protein that is involved with the transport of endosomes. That means that CHMP2B plays a role in the maturation of lysosomes and the integration of toxins into the lysosome. Therefore, CHMP2B may be a key gene in phagocytosis. This protein has a paralog with the human Snf7 gene, reinforcing the parallel resistance networks between Drosophila and humans (Capalbo et

al., 2012). However, not much else is known about CHMP2B, which merits more in-depth analysis and experimentation.

Lama is currently known to be involved in imaginal disc formation during development, as well as the formation of epithelial tubes during development (Table 1). Epithelial tubes are essential for the development of dorsal appendages. Research also shows that a link exists between dorsal formation (development) and immunity (Hoffman and Reichhart, 2002). More specifically, the protein known as Dorsal was at first discovered to be involved in the dorsoventral patterning of *Drosophila* embryos. However, Dorsal is also an immune factor (an NF-kB, more specifically), which is essential in the activation of genes involved in the Toll pathway of the innate immune response. It is possible that Lama can also be involved in immunity somehow, even though it is a developmental gene. However, where it fits in the immune response is unclear, and there is also a possibility that lama is not involved in resistance against fungal infection.

Two QTL were consistent with genes that encode immunoglobulins. Nothing is known about CG45263 besides the fact that its secondary structure suggests an immunoglobulin fold. In Drosophila, immunoglobulins are located on the surface of cells and are involved with cellular recognition. Research has shown that immunoglobulins are involved with neuronal development and recognition (Furukawa et al., 1992; Al-Anzi and Wyman, 2009). However, there also exists the immunoglobulin gene DSCAM, which allows for over 18,000 splice isoforms (Ferrandon et al., 2007). This immunoglobulin exists on the outside of phagocytes and contributes to the diversity of specificity among phagocytes. It is possible then that CG45263 has a role in cellular recognition of fungal pathogens. Dpr2 is also an immunoglobulin, though it has a currently defined role in sensory perception. Nevertheless, previous research and my QTL suggest a greater link between neural pathways and immune pathways. These candidate genes may provide the first step of discovering to what extent these two systems interact.

The next step in my experiment is to measure resistance for more RILs and to increase the number of replicates for what I have so far. With 3 replicates of ~300 RILs, I should have enough data to perform more detailed and specified QTL analyses that take variation between replicates, position of cages in the incubator that the flies were maintained in, sex into account. With a larger sample and replicate size my QTLs will be more accurate and have smaller confidence intervals. With a definitive analysis, it is up to the rest of the *Drosophila* community to do more detailed experiments, testing the effects that each candidate gene has on resistance and immunity as a whole. These include genetic and biochemical trials.

My project aimed to answer the broader scientific question of, "Which genes or genetic regions affect resistance against fungal infection?" The greater question that was asked also wanted to include genes that are not obviously linked with immunity, such as regulatory or housekeeping genes with other primary functions. But another important and related question has been asked: how do genes change to improve resistance against a fungal pathogen? This specific question could be answered by performing an artificial selection experiment. This has been performed by a colleague through an evolve and resequence method (Yonathan Estrella, Cornell University). By selecting for flies with better survival against *B.bassiana*, changes in the genome over many generations can be analyzed. So far, research has shown that the populations used in the experiment are evolving resistance against *B. bassiana*. The selection experiment is more likely to find genomic regions that have small effects on the resistance phenotype and play a role in adaptive immunity, whereas my experiment tends to find loci with big effects. However, my experiment also allows me to find alleles of low frequency.

For this genome scan experiment, the males and females showed differences in survival within and across RILs (Figure 5). This data is consistent with previous experiments performed with *Drosophila* and bacterial pathogens (Duneau et al., in preparation). Currently, another colleague is investigating the sexual dimorphism in response to *B. bassiana* in *D. melanogaster* in further detail

(Glen Malaret, Honors Thesis 2015). The dimorphism experiment observes the survival phenotypes of flies with mutations in different genes that we know are involved in the well-studied immune pathways of flies. The results from this project have shown that both the Toll and IMD innate immune pathways in flies are involved in explaining the difference in survival phenotype between males and females. This provides a better understanding of the genetic differences between male and female in terms of immune expression, which improves our current understandings on immunity. The combination of these three experiments will give us a detailed understanding of the genetic factors that affect resistance phenotypes. It will also serve to find new genes that biologists have never considered to be involved in immunity. The QTL analysis that I performed was basic and did not take sex differences into account. The survival per sex would also need to be factored into a more definitive QTL analysis, so that the X chromosome can be mapped more accurately.

In conclusion, the DSPR provides a large number of fly lines with both phenotypic and genetic diversity. The DSPR can be used to perform genomic analyses with a high level of resolution and accuracy. Basic QTL analysis with just 22 RILs provided many candidate genes, many of which have known functions in the innate immune response. Some genes show promise of a link between neural regulation and immune pathways. However, the significance of these genes in survival during infection needs to be explored.

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Appendix:

R Code For QTL analysis of 22 RILs:

```
R version 3.1.3 (2015-03-09) -- "Smooth Sidewalk"
Copyright (C) 2015 The R Foundation for Statistical Computing
Platform: x86_64-apple-darwin13.4.0 (64-bit)

R is free software and comes with ABSOLUTELY NO WARRANTY.
You are welcome to redistribute it under certain conditions.
Type 'license()' or 'licence()' for distribution details.

  Natural language support but running in an English locale

R is a collaborative project with many contributors.
Type 'contributors()' for more information and
'citation()' on how to cite R or R packages in publications.

Type 'demo()' for some demos, 'help()' for on-line help, or
'help.start()' for an HTML browser interface to help.
Type 'q()' to quit R.

[R.app GUI 1.65 (6913) x86_64-apple-darwin13.4.0]

[History restored from /Users/kgar/.Rapp.history]

> setwd('/Users/kgar/Downloads')
starting httpd help server ... done
> mydata <- read.table("data1.csv", header=TRUE, sep=",")
> scan <- DSPRscan(survival ~1, design = "inbredA", phenotype.dat = mydata, id.col='patRIL')
> peaks <- DSPRpeaks(scan, method = 'both', threshold = 6.8, LODdrop = 11, BCIprob = .99)
> peaks
[[1]]
[[1]]$threshold
[1] 6.8

[[1]]$peak
  chr   Ppos      Gpos      LOD
1  3L 880000 0.1764202 7.033538

[[1]]$LODdrop
[1] 11

[[1]]$BCIprob
[1] 0.99

[[1]]$CI
[[1]]$CI$LODdrop
          chr     Ppos          Gpos      LOD
```

```
Lower Bound   X   160000 -1.700662e-08 1.039640
Upper Bound  3R 27840000  1.029947e+02 1.892524

[[1]]$CI$BCI
          chr    Ppos         Gpos      LOD
Lower Bound   3  190000 3.728055e-06 4.112092
Upper Bound   3 2940000 5.151259e+00 4.506314


[[1]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
       0       11        2        5        1        1        0
      A8     Hets Uncertain
       0        0        2

[[1]]$geno.means
        Estimate   Std. Error
AA1  3165.8732230 1.712602e+03
AA2     0.6932238 2.843687e-02
AA3     0.6929307 5.899971e-02
AA4     0.4769530 5.411338e-02
AA5     0.6898329 9.427106e-02
AA6     0.4190212 9.427151e-02
AA7 -8188.2588395 3.452052e+03
AA8 35789.7473287 2.349862e+04

[[1]]$perct.var
[1] 77.06032

[[1]]$entropy
[1] 0.01508719


[[2]]
[[2]]$threshold
[1] 6.8

[[2]]$peak
  chr    Ppos     Gpos      LOD
2  3L 5120000 12.97089 7.845522

[[2]]$LODdrop
[1] 11

[[2]]$BCIprob
[1] 0.99

[[2]]$CI
[[2]]$CI$LODdrop
          chr     Ppos         Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.039640
```

```
Upper Bound  3R 27840000  1.029947e+02 1.892524

[[2]]$CI$BCI
            chr    Ppos     Gpos      LOD
Lower Bound   3 5040000 12.72700 7.385966
Upper Bound   3 5570000 14.29497 8.006461


[[2]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
       2        1       11        2        2        0        0
      A8     Hets Uncertain
       0        0        4

[[2]]$geno.means
       Estimate  Std. Error
AA1    0.8186656  0.05527984
AA2    0.3508204  0.07817527
AA3    0.6477345  0.02344428
AA4    0.7089579  0.05695187
AA5    0.7014977  0.05490988
AA6   -0.1813907  0.16440854
AA7    0.6671303  0.08240250
AA8 -145.5080439 67.54410012

[[2]]$perct.var
[1] 80.646

[[2]]$entropy
[1] 0.02867327


[[3]]
[[3]]$threshold
[1] 6.8

[[3]]$peak
  chr    Ppos     Gpos      LOD
3  3L 5350000 13.65581 8.076566

[[3]]$LODdrop
[1] 11

[[3]]$BCIprob
[1] 0.99

[[3]]$CI
[[3]]$CI$LODdrop
            chr     Ppos          Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.039640
Upper Bound  3R 27840000  1.029947e+02 1.892524
```

```
[[3]]$CI$BCI
          chr    Ppos     Gpos      LOD
Lower Bound   3 5040000 12.72700 7.385966
Upper Bound   3 5570000 14.29497 8.006461


[[3]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
       2        1       12        0        3        0        0
      A8     Hets Uncertain
       0        0        4

[[3]]$geno.means
       Estimate   Std. Error
AA1    1.78438005   0.90775887
AA2    0.35171194   0.07634067
AA3    0.64570152   0.02248762
AA4    0.06031363   4.77064868
AA5    0.74971398   0.06999579
AA6   -0.48390263   0.27814837
AA7    0.73521185   0.16274771
AA8 -764.17010993 717.77872725

[[3]]$perct.var
[1] 81.55975

[[3]]$entropy
[1] 0.0486885


[[4]]
[[4]]$threshold
[1] 6.8

[[4]]$peak
  chr    Ppos     Gpos      LOD
4  3L 5370000 13.71443 8.140847

[[4]]$LODdrop
[1] 11

[[4]]$BCIprob
[1] 0.99

[[4]]$CI
[[4]]$CI$LODdrop
          chr     Ppos          Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.039640
Upper Bound  3R 27840000  1.029947e+02 1.892524
```

```
[[4]]$CI$BCI
           chr    Ppos     Gpos      LOD
Lower Bound   3 5040000 12.72700 7.385966
Upper Bound   3 5570000 14.29497 8.006461


[[4]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
       2        1       11        0        3        0        0
      A8     Hets Uncertain
       0        0        5

[[4]]$geno.means
     Estimate  Std. Error
AA1  0.8333148  0.06123747
AA2  0.3507532  0.07577700
AA3  0.6370151  0.02219672
AA4  2.1810285  1.35109229
AA5  0.6834229  0.04629276
AA6 -0.7266278  0.27445112
AA7  0.7038444  0.17022786
AA8 -7.5983269 20.01500594

[[4]]$perct.var
[1] 81.80622

[[4]]$entropy
[1] 0.0543435


[[5]]
[[5]]$threshold
[1] 6.8

[[5]]$peak
  chr    Ppos     Gpos      LOD
5  3L 5560000 14.26613 8.048203

[[5]]$LODdrop
[1] 11

[[5]]$BCIprob
[1] 0.99

[[5]]$CI
[[5]]$CI$LODdrop
           chr     Ppos          Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.039640
Upper Bound  3R 27840000  1.029947e+02 1.892524

[[5]]$CI$BCI
```

```
          chr    Ppos     Gpos      LOD
Lower Bound   3 5040000 12.72700 7.385966
Upper Bound   3 5570000 14.29497 8.006461


[[5]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
       0        1       14        1        2        0        0
      A8     Hets Uncertain
       0        0        4

[[5]]$geno.means
       Estimate  Std. Error
AA1   -6.1922188 22.65204082
AA2    0.3513399  0.07650534
AA3    0.6395078  0.02210231
AA4    0.9363562  0.07341581
AA5    0.7100951  0.05457949
AA6 -122.0203518 25.18038616
AA7    4.6110856 12.04279034
AA8  260.0961168 54.12440201

[[5]]$perct.var
[1] 81.44995

[[5]]$entropy
[1] 0.04984429


[[6]]
[[6]]$threshold
[1] 6.8

[[6]]$peak
  chr     Ppos     Gpos      LOD
6  3L 13700000 40.52595 8.065118

[[6]]$LODdrop
[1] 11

[[6]]$BCIprob
[1] 0.99

[[6]]$CI
[[6]]$CI$LODdrop
          chr     Ppos          Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.039640
Upper Bound  3R 27840000  1.029947e+02 1.892524

[[6]]$CI$BCI
          chr     Ppos     Gpos      LOD
```

```
Lower Bound   3 13370000 39.95766 4.301614
Upper Bound   3 17550000 44.61522 4.286417


[[6]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
       2        1       11        6        1        0        1
      A8     Hets Uncertain
       0        0        0

[[6]]$geno.means
        Estimate   Std. Error
AA1     0.5364496 8.682063e-02
AA2     0.2553735 9.771073e-02
AA3     0.6354571 3.295011e-02
AA4     0.6636295 4.582103e-02
AA5     0.8694465 9.772302e-02
AA6 -5167.1531903 1.886922e+03
AA7     0.6934332 9.770680e-02
AA8 62791.7801013 7.380982e+04

[[6]]$perct.var
[1] 81.51551

[[6]]$entropy
[1] 8.415936e-05


[[7]]
[[7]]$threshold
[1] 6.8

[[7]]$peak
  chr    Ppos     Gpos      LOD
7  3R 8370000 52.04484 7.229279

[[7]]$LODdrop
[1] 11

[[7]]$BCIprob
[1] 0.99

[[7]]$CI
[[7]]$CI$LODdrop
           chr     Ppos          Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.039640
Upper Bound  3R 27840000  1.029947e+02 1.892524

[[7]]$CI$BCI
           chr    Ppos     Gpos      LOD
Lower Bound   3 8370000 52.04484 7.229279
```

```
Upper Bound   3 9280000 53.41437 6.966367


[[7]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
       0        1        2       17        0        0        1
      A8     Hets Uncertain
       0        0        1

[[7]]$geno.means
       Estimate   Std. Error
AA1 -6.710313e+05 5.602880e+05
AA2 -2.817642e-01 7.771968e-01
AA3  1.077857e+00 2.597589e-01
AA4  8.562951e-01 2.105849e-01
AA5 -2.044934e+05 6.051216e+04
AA6  2.962590e+06 2.519753e+06
AA7  8.398108e-01 3.965104e-01
AA8 -4.116035e+05 3.909062e+05

[[7]]$perct.var
[1] 77.98124

[[7]]$entropy
[1] 0.008102559


[[8]]
[[8]]$threshold
[1] 6.8

[[8]]$peak
  chr    Ppos    Gpos      LOD
8  3R 8970000 52.9342 7.428952

[[8]]$LODdrop
[1] 11

[[8]]$BCIprob
[1] 0.99

[[8]]$CI
[[8]]$CI$LODdrop
          chr     Ppos         Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.039640
Upper Bound  3R 27840000  1.029947e+02 1.892524

[[8]]$CI$BCI
          chr    Ppos     Gpos      LOD
Lower Bound   3 8370000 52.04484 7.229279
Upper Bound   3 9280000 53.41437 6.966367
```

```
[[8]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
       0        1        3       17        0        0        1
      A8     Hets Uncertain
       0        0        0

[[8]]$geno.means
       Estimate   Std. Error
AA1  1.198405e+04 4.111655e+04
AA2  5.803232e-01 9.179520e-02
AA3  7.905815e-01 5.953452e-02
AA4  6.572630e-01 2.649576e-02
AA5 -1.627998e+04 5.038278e+03
AA6  3.747687e+04 2.468271e+04
AA7  4.210636e-01 8.905944e-02
AA8 -5.225874e+04 3.693686e+04

[[8]]$perct.var
[1] 78.88259

[[8]]$entropy
[1] 0.000186188


[[9]]
[[9]]$threshold
[1] 6.8

[[9]]$peak
  chr    Ppos    Gpos      LOD
9  3R 8990000 52.9648 6.825123

[[9]]$LODdrop
[1] 11

[[9]]$BCIprob
[1] 0.99

[[9]]$CI
[[9]]$CI$LODdrop
           chr     Ppos          Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.039640
Upper Bound  3R 27840000  1.029947e+02 1.892524

[[9]]$CI$BCI
           chr    Ppos     Gpos      LOD
Lower Bound   3 8370000 52.04484 7.229279
Upper Bound   3 9280000 53.41437 6.966367
```

```
[[9]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
       0        1        3       17        0        0        1
      A8     Hets Uncertain
       0        0        0

[[9]]$geno.means
       Estimate   Std. Error
AA1 -1.170401e+04 3.382062e+04
AA2  6.011429e-01 8.988828e-02
AA3  7.793262e-01 5.759896e-02
AA4  6.577657e-01 2.687140e-02
AA5 -7.834171e+03 2.330717e+03
AA6  1.918516e+04 2.228876e+04
AA7  4.221804e-01 8.803185e-02
AA8 -1.976058e+04 2.970806e+04

[[9]]$perct.var
[1] 76.03738

[[9]]$entropy
[1] 0.0001835194


[[10]]
[[10]]$threshold
[1] 6.8

[[10]]$peak
   chr    Ppos     Gpos      LOD
10  3R 9010000 52.99546 6.925887

[[10]]$LODdrop
[1] 11

[[10]]$BCIprob
[1] 0.99

[[10]]$CI
[[10]]$CI$LODdrop
           chr     Ppos         Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.039640
Upper Bound  3R 27840000  1.029947e+02 1.892524

[[10]]$CI$BCI
           chr    Ppos     Gpos      LOD
Lower Bound   3 8370000 52.04484 7.229279
Upper Bound   3 9280000 53.41437 6.966367
```

```
[[10]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
       0        1        3       17        0        0        1
      A8     Hets Uncertain
       0        0        0

[[10]]$geno.means
       Estimate   Std. Error
AA1 -1.905189e+04 3.105368e+04
AA2  6.089487e-01 8.941484e-02
AA3  7.748465e-01 5.618068e-02
AA4  6.589538e-01 2.645710e-02
AA5 -5.164253e+03 1.500092e+03
AA6  1.403119e+04 2.097487e+04
AA7  4.224461e-01 8.766345e-02
AA8 -1.294811e+04 2.794685e+04

[[10]]$perct.var
[1] 76.53752

[[10]]$entropy
[1] 0.0001798194


[[11]]
[[11]]$threshold
[1] 6.8

[[11]]$peak
   chr    Ppos     Gpos      LOD
11  3R 9050000 53.05693 6.806121

[[11]]$LODdrop
[1] 11

[[11]]$BCIprob
[1] 0.99

[[11]]$CI
[[11]]$CI$LODdrop
           chr     Ppos          Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.039640
Upper Bound  3R 27840000  1.029947e+02 1.892524

[[11]]$CI$BCI
           chr    Ppos     Gpos      LOD
Lower Bound   3 8370000 52.04484 7.229279
Upper Bound   3 9280000 53.41437 6.966367


[[11]]$founderNs
```

```
      A1       A2       A3       A4       A5       A6       A7
       0        1        3       17        0        0        1
      A8     Hets Uncertain
       0        0        0

[[11]]$geno.means
        Estimate   Std. Error
AA1 -3.936880e+04 4.740660e+04
AA2  6.274719e-01 9.279845e-02
AA3  7.681436e-01 5.325246e-02
AA4  6.631340e-01 2.588445e-02
AA5 -3.085823e+03 8.758422e+02
AA6  1.070430e+04 1.924887e+04
AA7  4.240835e-01 8.747383e-02
AA8 -1.199979e+04 2.974494e+04

[[11]]$perct.var
[1] 75.94188

[[11]]$entropy
[1] 0.0001636971


[[12]]
[[12]]$threshold
[1] 6.8

[[12]]$peak
   chr    Ppos     Gpos      LOD
12  3R 9100000 53.13407 7.280563

[[12]]$LODdrop
[1] 11

[[12]]$BCIprob
[1] 0.99

[[12]]$CI
[[12]]$CI$LODdrop
            chr     Ppos          Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.039640
Upper Bound  3R 27840000  1.029947e+02 1.892524

[[12]]$CI$BCI
            chr    Ppos     Gpos      LOD
Lower Bound   3 8370000 52.04484 7.229279
Upper Bound   3 9280000 53.41437 6.966367


[[12]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
```

```
      0       1       3       17       0       0       1
     A8     Hets Uncertain
      0       0       0

[[12]]$geno.means
       Estimate   Std. Error
AA1  1.018265e+06 1.269133e+06
AA2  2.792957e-01 4.164800e-01
AA3  5.463576e-01 2.689833e-01
AA4  6.467249e-01 3.241717e-02
AA5 -2.390798e+03 4.718685e+02
AA6 -4.472747e+04 6.066813e+04
AA7  4.132317e-01 8.852987e-02
AA8  1.314866e+05 1.732930e+05

[[12]]$perct.var
[1] 78.21635

[[12]]$entropy
[1] 0.0001492037


[[13]]
[[13]]$threshold
[1] 6.8

[[13]]$peak
   chr    Ppos    Gpos      LOD
13  3R 9170000 53.2426 7.720121

[[13]]$LODdrop
[1] 11

[[13]]$BCIprob
[1] 0.99

[[13]]$CI
[[13]]$CI$LODdrop
          chr     Ppos         Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.039640
Upper Bound  3R 27840000  1.029947e+02 1.892524

[[13]]$CI$BCI
          chr    Ppos     Gpos      LOD
Lower Bound   3 8370000 52.04484 7.229279
Upper Bound   3 9280000 53.41437 6.966367


[[13]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
       0       1        2       17        0        0        1
```

```
      A8      Hets Uncertain
       0        0         1

[[13]]$geno.means
        Estimate   Std. Error
AA1  8.299012e+04 1.829560e+04
AA2  8.428789e-01 1.853836e-01
AA3  7.514432e-01 7.259613e-02
AA4  6.615586e-01 2.520331e-02
AA5 -1.964127e+03 3.707881e+02
AA6  3.442228e+04 3.755118e+04
AA7  4.871206e-01 9.875141e-02
AA8 -2.203174e+05 1.797571e+05

[[13]]$perct.var
[1] 80.13124

[[13]]$entropy
[1] 0.005146264


[[14]]
[[14]]$threshold
[1] 6.8

[[14]]$peak
   chr    Ppos     Gpos      LOD
14  3R 9190000 53.27372 8.550025

[[14]]$LODdrop
[1] 11

[[14]]$BCIprob
[1] 0.99

[[14]]$CI
[[14]]$CI$LODdrop
           chr     Ppos         Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.039640
Upper Bound  3R 27840000  1.029947e+02 1.892524

[[14]]$CI$BCI
           chr    Ppos     Gpos      LOD
Lower Bound   3 8370000 52.04484 7.229279
Upper Bound   3 9280000 53.41437 6.966367


[[14]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
       0        1        2       17        0        0        1
      A8      Hets Uncertain
```

```
      0       0       1

[[14]]$geno.means
       Estimate   Std. Error
AA1  3.154241e+04 6.647678e+03
AA2  8.286681e-01 2.254269e-01
AA3  8.057423e-01 1.218318e-01
AA4  6.636979e-01 2.520697e-02
AA5 -2.497580e+03 4.613063e+02
AA6  4.172175e+04 4.552469e+04
AA7  5.445783e-01 1.453785e-01
AA8 -2.009551e+05 1.964494e+05

[[14]]$perct.var
[1] 83.29967

[[14]]$entropy
[1] 0.008343012


[[15]]
[[15]]$threshold
[1] 6.8

[[15]]$peak
   chr    Ppos     Gpos     LOD
15  3R 9210000 53.30489 8.26639

[[15]]$LODdrop
[1] 11

[[15]]$BCIprob
[1] 0.99

[[15]]$CI
[[15]]$CI$LODdrop
          chr     Ppos         Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.039640
Upper Bound  3R 27840000  1.029947e+02 1.892524

[[15]]$CI$BCI
          chr    Ppos     Gpos      LOD
Lower Bound   3 8370000 52.04484 7.229279
Upper Bound   3 9280000 53.41437 6.966367


[[15]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
       0        1        2       17        0        0        1
      A8     Hets Uncertain
       0        0        1
```

```
[[15]]$geno.means
        Estimate   Std. Error
AA1  2.025740e+04 4.082154e+03
AA2  9.061013e-01 2.707940e-01
AA3  9.006433e-01 1.850096e-01
AA4  6.668743e-01 2.514379e-02
AA5 -3.357815e+03 5.942743e+02
AA6  6.235127e+04 5.618702e+04
AA7  6.466683e-01 2.095059e-01
AA8 -2.523116e+05 2.161907e+05

[[15]]$perct.var
[1] 82.27811

[[15]]$entropy
[1] 0.008851761


[[16]]
[[16]]$threshold
[1] 6.8

[[16]]$peak
   chr    Ppos     Gpos      LOD
16  3R 9230000 53.33611 9.278923

[[16]]$LODdrop
[1] 11

[[16]]$BCIprob
[1] 0.99

[[16]]$CI
[[16]]$CI$LODdrop
           chr     Ppos          Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.039640
Upper Bound  3R 27840000  1.029947e+02 1.892524

[[16]]$CI$BCI
           chr    Ppos     Gpos      LOD
Lower Bound   3 8370000 52.04484 7.229279
Upper Bound   3 9280000 53.41437 6.966367


[[16]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
       0        1        2       17        0        0        1
      A8     Hets Uncertain
       0        0        1
```

```
[[16]]$geno.means
        Estimate   Std. Error
AA1  1.654994e+04 2.873751e+03
AA2  1.004245e+00 3.086882e-01
AA3  1.029788e+00 2.512451e-01
AA4  6.712201e-01 2.506223e-02
AA5 -4.928170e+03 8.173857e+02
AA6  9.632085e+04 7.170479e+04
AA7  7.848628e-01 2.782639e-01
AA8 -3.246426e+05 2.364262e+05

[[16]]$perct.var
[1] 85.66289

[[16]]$entropy
[1] 0.00694693


[[17]]
[[17]]$threshold
[1] 6.8

[[17]]$peak
   chr    Ppos     Gpos      LOD
17  3R 9280000 53.41437 6.966367

[[17]]$LODdrop
[1] 11

[[17]]$BCIprob
[1] 0.99

[[17]]$CI
[[17]]$CI$LODdrop
          chr     Ppos          Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.039640
Upper Bound  3R 27840000  1.029947e+02 1.892524

[[17]]$CI$BCI
          chr    Ppos     Gpos      LOD
Lower Bound   3 8370000 52.04484 7.229279
Upper Bound   3 9280000 53.41437 6.966367


[[17]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
       0        1        2       18        0        0        1
      A8     Hets Uncertain
       0        0        0

[[17]]$geno.means
```

```
        Estimate   Std. Error
AA1  8.352595e+04 9.018363e+04
AA2  1.555012e+00 6.383041e-01
AA3  1.612461e+00 6.473533e-01
AA4  8.099249e-01 1.174587e-01
AA5 -1.946771e+04 3.609312e+03
AA6  3.109818e+05 2.449750e+05
AA7  1.493258e+00 7.202764e-01
AA8 -8.460084e+05 5.817749e+05

[[17]]$perct.var
[1] 76.73549

[[17]]$entropy
[1] 5.902176e-05


attr(,"class")
[1] "peaks"
> main.peak <- peaks[[7]]
> peakChr <- main.peak$peak$chr
> peakPos <- main.peak$peak$Ppos
> peak.int <- LocalInt(peakChr, peakPos, phenotype.dat = mydata, pheno.name = "survival", design =
"inbredA")
> peak.int
    chr    Ppos     Gpos        LOD
1    3R 7370000 50.72354 0.03987994
2    3R 7380000 50.73552 0.03990814
3    3R 7390000 50.74753 0.03992709
4    3R 7400000 50.75956 0.03992578
5    3R 7410000 50.77163 0.03992352
6    3R 7420000 50.78372 0.03992212
7    3R 7430000 50.79584 0.03993207
8    3R 7440000 50.80798 0.03992713
9    3R 7450000 50.82016 0.03992219
10   3R 7460000 50.83236 0.03991725
11   3R 7470000 50.84458 0.13296374
12   3R 7480000 50.85684 0.59584266
13   3R 7490000 50.86912 0.91908168
14   3R 7500000 50.88142 1.08136661
15   3R 7510000 50.89376 1.09771169
16   3R 7520000 50.90612 1.08225778
17   3R 7530000 50.91850 1.08232193
18   3R 7540000 50.93092 1.08240473
19   3R 7550000 50.94336 1.08248964
20   3R 7560000 50.95582 1.08257280
21   3R 7570000 50.96832 1.08265704
22   3R 7580000 50.98084 1.08274027
23   3R 7590000 50.99338 1.08282478
24   3R 7600000 51.00595 1.08290702
25   3R 7610000 51.01855 1.08296984
```

26	3R	7620000	51.03117	1.08302331
27	3R	7630000	51.04382	1.08307849
28	3R	7640000	51.05649	1.08313296
29	3R	7650000	51.06919	1.08319062
30	3R	7660000	51.08192	1.08329541
31	3R	7670000	51.09467	1.08332606
32	3R	7680000	51.10744	1.08335458
33	3R	7690000	51.12024	1.08338481
34	3R	7700000	51.13307	1.08341377
35	3R	7710000	51.14592	1.08344291
36	3R	7720000	51.15880	1.08347045
37	3R	7730000	51.17170	1.08350152
38	3R	7740000	51.18462	1.08352864
39	3R	7750000	51.19757	1.08355704
40	3R	7760000	51.21055	1.08628954
41	3R	7770000	51.22355	1.09384110
42	3R	7780000	51.23657	1.10172273
43	3R	7790000	51.24962	1.10994508
44	3R	7800000	51.26269	1.11851674
45	3R	7810000	51.27579	1.12747573
46	3R	7820000	51.28891	1.13611043
47	3R	7830000	51.30205	1.14496177
48	3R	7840000	51.31522	1.15422558
49	3R	7850000	51.32841	1.16391738
50	3R	7860000	51.34163	1.17408024
51	3R	7870000	51.35487	1.18552169
52	3R	7880000	51.36813	1.19743929
53	3R	7890000	51.38142	1.20981343
54	3R	7900000	51.39473	1.22261789
55	3R	7910000	51.40806	1.23620763
56	3R	7920000	51.42141	1.25018608
57	3R	7930000	51.43479	1.26444920
58	3R	7940000	51.44819	1.27892208
59	3R	7950000	51.46162	1.29352907
60	3R	7960000	51.47507	1.30818834
61	3R	7970000	51.48854	1.32282050
62	3R	7980000	51.50203	1.33735503
63	3R	7990000	51.51554	1.35171981
64	3R	8000000	51.52908	1.36585176
65	3R	8010000	51.54264	1.37968937
66	3R	8020000	51.55622	1.39318077
67	3R	8030000	51.56983	1.40627266
68	3R	8040000	51.58345	1.41891379
69	3R	8050000	51.59710	1.43105996
70	3R	8060000	51.61077	1.44267907
71	3R	8070000	51.62446	1.45370489
72	3R	8080000	51.63817	1.46410874
73	3R	8090000	51.65191	1.47384258
74	3R	8100000	51.66566	1.48286840
75	3R	8110000	51.67944	1.49113848
76	3R	8120000	51.69324	1.49860533

77	3R	8130000	51.70706	1.50522141
78	3R	8140000	51.72090	1.51093055
79	3R	8150000	51.73476	1.51571189
80	3R	8160000	51.74864	1.51946069
81	3R	8170000	51.76255	1.52204883
82	3R	8180000	51.77647	1.52338666
83	3R	8190000	51.79042	1.52336922
84	3R	8200000	51.80438	1.52188091
85	3R	8210000	51.81837	1.51878581
86	3R	8220000	51.83237	1.51392623
87	3R	8230000	51.84640	1.50706894
88	3R	8240000	51.86045	1.49799327
89	3R	8250000	51.87451	1.48644550
90	3R	8260000	51.88860	1.47212673
91	3R	8270000	51.90271	1.45453179
92	3R	8280000	51.91683	1.43316323
93	3R	8290000	51.93098	1.40780699
94	3R	8300000	51.94514	1.37812871
95	3R	8310000	51.95933	1.34402327
96	3R	8320000	51.97353	1.30576787
97	3R	8330000	51.98776	1.26420093
98	3R	8340000	52.00200	1.22061763
99	3R	8350000	52.01626	1.19899627
100	3R	8360000	52.03054	1.19201259
101	3R	8370000	52.04484	1.18624896
102	3R	8380000	52.05916	1.18168968
103	3R	8390000	52.07350	1.17823773
104	3R	8400000	52.08785	1.17581579
105	3R	8410000	52.10223	1.17438420
106	3R	8420000	52.11662	1.17379994
107	3R	8430000	52.13103	1.17396314
108	3R	8440000	52.14546	1.17417456
109	3R	8450000	52.15991	1.17418072
110	3R	8460000	52.17438	1.17417768
111	3R	8470000	52.18886	1.17417377
112	3R	8480000	52.20337	1.17417072
113	3R	8490000	52.21789	1.17416912
114	3R	8500000	52.23242	1.17417297
115	3R	8510000	52.24698	1.17416945
116	3R	8520000	52.26155	1.17416848
117	3R	8530000	52.27614	1.17416894
118	3R	8540000	52.29075	1.17416390
119	3R	8550000	52.30538	1.17416905
120	3R	8560000	52.32002	1.17417420
121	3R	8570000	52.33468	1.17417501
122	3R	8580000	52.34936	1.17417865
123	3R	8590000	52.36405	1.17418261
124	3R	8600000	52.37876	1.17417961
125	3R	8610000	52.39349	1.17418356
126	3R	8620000	52.40824	1.17418231
127	3R	8630000	52.42300	1.17418496

128	3R	8640000	52.43777	1.17418414
129	3R	8650000	52.45257	1.17418661
130	3R	8660000	52.46738	1.17418948
131	3R	8670000	52.48221	1.17418826
132	3R	8680000	52.49705	1.17418574
133	3R	8690000	52.51191	1.17419016
134	3R	8700000	52.52678	1.17418807
135	3R	8710000	52.54168	1.17418729
136	3R	8720000	52.55658	1.17418954
137	3R	8730000	52.57151	1.17418875
138	3R	8740000	52.58645	1.17418883
139	3R	8750000	52.60140	1.17418935
140	3R	8760000	52.61637	1.17419074
141	3R	8770000	52.63136	1.17418821
142	3R	8780000	52.64636	1.17418731
143	3R	8790000	52.66137	1.17421014
144	3R	8800000	52.67641	1.17425469
145	3R	8810000	52.69145	1.17429699
146	3R	8820000	52.70652	1.17434016
147	3R	8830000	52.72159	1.17438290
148	3R	8840000	52.73668	1.17442478
149	3R	8850000	52.75179	1.17446796
150	3R	8860000	52.76691	1.17451029
151	3R	8870000	52.78205	1.17455350
152	3R	8880000	52.79720	1.17459542
153	3R	8890000	52.81237	1.17463883
154	3R	8900000	52.82755	1.17468080
155	3R	8910000	52.84274	1.17472451
156	3R	8920000	52.85795	1.17476606
157	3R	8930000	52.87317	1.17535706
158	3R	8940000	52.88841	1.17693741
159	3R	8950000	52.90366	1.17785559
160	3R	8960000	52.91893	1.17819371
161	3R	8970000	52.93420	1.17831539
162	3R	8980000	52.94950	1.17843842
163	3R	8990000	52.96480	1.17856234
164	3R	9000000	52.98012	1.17868457
165	3R	9010000	52.99546	1.17880769
166	3R	9020000	53.01081	1.17893085
167	3R	9030000	53.02617	1.17905535
168	3R	9040000	53.04154	1.17916608
169	3R	9050000	53.05693	1.17927549
170	3R	9060000	53.07233	1.17938494
171	3R	9070000	53.08774	1.17949541
172	3R	9080000	53.10317	1.17959345
173	3R	9090000	53.11861	1.17969124
174	3R	9100000	53.13407	1.17978903
175	3R	9110000	53.14953	1.17988597
176	3R	9120000	53.16501	1.17998463
177	3R	9130000	53.18050	1.18008748
178	3R	9140000	53.19601	1.18018779

```
179  3R 9150000 53.21153 1.18029165
180  3R 9160000 53.22706 1.17145885
181  3R 9170000 53.24260 1.13156047
182  3R 9180000 53.25815 1.08801659
183  3R 9190000 53.27372 1.04017992
184  3R 9200000 53.28930 0.98729191
185  3R 9210000 53.30489 0.92853105
186  3R 9220000 53.32049 0.86328477
187  3R 9230000 53.33611 0.79195765
188  3R 9240000 53.35174 0.71804208
189  3R 9250000 53.36738 0.65027342
190  3R 9260000 53.38303 0.64064571
191  3R 9270000 53.39869 0.64052624
192  3R 9280000 53.41437 0.64048294
193  3R 9290000 53.43005 0.64043903
194  3R 9300000 53.44575 0.64038564
195  3R 9310000 53.46146 0.64033269
196  3R 9320000 53.47719 0.64027931
197  3R 9330000 53.49292 0.64022551
198  3R 9340000 53.50866 0.64017345
199  3R 9350000 53.52442 0.64012313
200  3R 9360000 53.54019 0.64009496
201  3R 9370000 53.55596 0.64010115
> DSPRplot(list(scan), threshold=6.8)
```

R Code for QTL analysis for 125 RILs

```
> setwd('/Users/kgar/Downloads')
starting httpd help server ... done
> mydata <- read.table("data3.csv", header=TRUE, sep=",")
> scan <- DSPRscan(survival ~1, design = "inbredA", phenotype.dat = mydata, id.col='patRIL')
> perm <- DSPRperm(survival ~ 1, design = "inbredA", phenotype.dat = mydata, id.col='patRIL', alpha =
0.05)
> perm
$maxLODs
  [1]  5.513623  6.626438  7.686312  7.784792  5.651305  6.919108  5.951520
  [8]  6.846985  6.572547  5.973577  7.503696  5.060458  7.540894  6.933971
 [15]  7.305150  6.540588  6.826141  6.994460  6.860019  5.127516  6.670023
 [22]  5.942411  6.074481  6.604447  5.752864  7.899581  7.093060  8.210488
 [29]  9.372025  5.932358  5.842603  8.198210  6.278369  8.606489  5.222193
 [36]  5.523289  6.243968  6.051260  6.318168  6.310705  7.892143  6.876779
 [43]  5.516751  7.026889  8.595035  5.739334  5.468824  6.797593  8.715905
 [50]  7.786094  7.245167  7.866198  6.312805  7.273973  7.312956  7.667975
 [57]  6.039501  6.959684  6.258116  8.631747  7.022162  7.642303  5.300591
 [64]  6.162897  7.082869  6.744778  6.947308  5.120741  5.683847  5.161278
 [71]  7.520980  5.439202  7.227028  5.199832  6.894988  5.969463  6.987205
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```

```
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 [995] 5.548786 6.609051 6.116240 6.607726 5.773016 7.310937

$alpha
[1] 0.05

$threshold
    95%
8.478929

attr(,"class")
[1] "pt"
> peaks <- DSPRpeaks(scan, method = 'both', threshold = 6.8, LODdrop = 8)
> peaks
[[1]]
[[1]]$threshold
[1] 6.8

[[1]]$peak
  chr     Ppos   Gpos      LOD
1  2L 10900000 42.456 7.040812

[[1]]$LODdrop
[1] 8

[[1]]$BCIprob
[1] 0.95
```

```
[[1]]$CI
[[1]]$CI$LODdrop
          chr     Ppos         Gpos      LOD
Lower Bound   X   160000 -1.700662e-08 1.679536
Upper Bound  3R 27840000  1.029947e+02 1.007223

[[1]]$CI$BCI
          chr     Ppos     Gpos      LOD
Lower Bound   2 10520000 41.01131 6.365132
Upper Bound   2 11120000 43.21031 6.288307


[[1]]$founderNs
      A1       A2       A3       A4       A5       A6       A7
       1        0        0       46       39       10        6
      A8     Hets Uncertain
      13        0       10

[[1]]$geno.means
     Estimate Std. Error
AA1  0.7263111 0.16254289
AA2 -9.6331610 4.06085670
AA3  1.0405467 0.16236795
AA4  0.5765623 0.02435721
AA5  0.6075414 0.02663319
AA6  0.5439557 0.05278342
AA7  0.6162183 0.06211061
AA8  0.4007459 0.04620902

[[1]]$perct.var
[1] 22.84803

[[1]]$entropy
[1] 0.02479398


attr(,"class")
[1] "peaks"
> DSPRplot(list(scan), threshold=6.8)
```

R Code for the two-way ANOVA test

```
> setwd('/Users/kgar/Downloads')
>mydata<-read.table("replicate2.csv", header=TRUE, sep=",", dec=".",na.strings=".")
>attach(mydata)
> names(mydata)
[1] "replicate" "survival"  "RIL"
>replicate<-as.factor(replicate)
>RIL<-as.factor(RIL)
> anova<-aov(survival~RIL+replicate+RIL:replicate,data = mydata)
>anova
```

```
Call:
  aov(formula = survival ~ RIL + replicate + RIL:replicate, data = mydata)

Terms:
  RIL replicate RIL:replicate Residuals
Sum of Squares  13252.791    11.419       577.740  1038.092
Deg. of Freedom        21         1            21        22

Residual standard error: 6.869205
Estimated effects may be unbalanced

>summary(anova)
                Df          Sum Sq       Mean Sq      F value     Pr(>F)
RIL             21           13253        631.1           13.374     3.79e-08 ***
replicate       1              11          11.4             0.242     0.628
RIL:replicate 21              578          27.5             0.583     0.889
Residuals       22            1038          47.2
---
  Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```